PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 04 May 2000 (04.05.00)	
International application No. PCT/SE99/01231	**Applicant's or agent's file reference** 4092 PCT
International filing date (day/month/year) 06 July 1999 (06.07.99)	**Priority date** (day/month/year) 13 July 1998 (13.07.98)
Applicant LARSSON, Cecilia et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

03 February 2000 (03.02.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer R. E. Stoffel
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

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REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For Receiving Office use only

International Application No. PCT/SE99/01231

International Filing Date 06-07-1999

The Swedish Patent Office
PCT International Application

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* 4092 PCT

Box No. I TITLE OF INVENTION

Material for bone reconstruction

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

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☐ This person is also inventor.

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State *(that is, country)* of nationality: SE

State *(that is, country)* of residence: SE

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

GS Development AB
Jägershillgatan 15
213 75 MALMÖ
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This person is:

☒ applicant only

☐ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: SE

State *(that is, country)* of residence: SE

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is ~~hereby~~/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

OLSSON, Gunnar
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☐ **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

06 -07- 1999

Continuation of Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

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This person is:
- [ ] applicant only
- [X] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: SE

State *(that is, country)* of residence: SE

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [X] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

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S-236 33 HÖLLVIKEN
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This person is:
- [ ] applicant only
- [X] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: SE

State *(that is, country)* of residence: SE

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [X] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [ ] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [ ] the United States of America only
- [ ] the States indicated in the Supplemental Box

- [ ] Further applicants and/or (further) inventors are indicated on another continuation sheet.

See Notes to the request form

Box No. V DESIGNATION OF STATES 06 -07- 1999

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one check-box must be marked)*:

Regional Patent

- ☒ AP **ARIPO Patent:** GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, SD Sudan, SZ Swaziland, UG Uganda, ZW Zimbabwe and any other State which is a Contracting State of the Harare Protocol and of the PCT
- ☒ EA **Eurasian Patent:** AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakhstan, MD Republic of Moldovia, RU Russian Federation, TJ Tajikistan, TM Turkmenistan and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT
- ☒ EP **European Patent:** AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, CY Cyprus, DE Germany, DK Denmark, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT
- ☒ OA **OAPI Patent:** BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, GW Guinea-Bissau, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* _____

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

☒	AL	Albania	☒	LS	Lesotho
☒	AM	Armenia	☒	LT	Lithuania
☒	AT	Austria +Utility Model	☒	LU	Luxembourg
☒	AU	Australia	☒	LV	Latvia
☒	AZ	Azerbaijan	☒	MD	Republic of Moldova
☒	BA	Bosnia and Herzegovina	☒	MG	Madagascar
☒	BB	Barbados	☒	MK	The former Yugoslav Republic of Macedonia
☒	BG	Bulgaria	☒	MN	Mongolia
☒	BR	Brazil	☒	MW	Malawi
☒	BY	Belarus	☒	MX	Mexico
☒	CA	Canada	☒	NO	Norway
☒	CH and LI	Switzerland and Liechtenstein	☒	NZ	New Zealand
☒	CN	China	☒	PL	Poland
☒	CU	Cuba	☒	PT	Portugal
☒	CZ	Czech Republic +Utility Model	☒	RO	Romania
☒	DE	Germany +Utility Model	☒	RU	Russian Federation
☒	DK	Denmark +Utility Model	☒	SD	Sudan
☒	EE	Estonia +Utility Model	☒	SE	Sweden
☒	ES	Spain	☒	SG	Singapore
☒	FI	Finland +Utility Model	☒	SI	Slovenia
☒	GB	United Kingdom	☒	SK	Slovakia +Utility Model
☒	GD	Grenada	☒	SL	Sierra Leone
☒	GE	Georgia	☒	TJ	Tajikistan
☒	GH	Ghana	☒	TM	Turkmenistan
☒	GM	Gambia	☒	TR	Turkey
☒	HR	Croatia	☒	TT	Trinidad and Tobago
☒	HU	Hungary	☒	UA	Ukraine
☒	ID	Indonesia	☒	UG	Uganda
☒	IL	Israel	☒	US	United States of America
☒	IN	India	☒	UZ	Uzbekistan
☒	IS	Iceland	☒	VN	Viet Nam
☒	JP	Japan	☒	YU	Yugoslavia
☒	KE	Kenya	☒	ZW	Zimbabve
☒	KG	Kyrgyzstan			
☒	KP	Democratic People's Republic of Korea			
☒	KR	Republic of Korea			
☒	KZ	Kazakhstan			
☒	LC	Saint Lucia			
☒	LK	Sri Lanka			
☒	LR	Liberia			

Check-boxes reserved for designating States (for the purposes of a national patent) which have become party to the PCT after issuance of this sheet:

- ☒ AE United Arab Emirates
- ☒ ZA South Africa
- ☐

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)*

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Box No. VI PRIORITY CL[illegible] ☐ Further prio[illegible]aims are indicated in the Supplemental Box.

Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is: national application: country	regional application:* regional Office	international application: receiving Office
item (1) 13/07/98 13 July 1998	98 02529-9	SE		
item (2)				
item (3)				

☒ The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office)* identified above as item(s): (1)

** Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.*

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used)*:	**Request to use results of earlier search; reference to that search** *(if an earlier search has been carried out by or requested from the International Searching Authority)*: Date *(day/month/year)*	Number	Country *(or regional Office)*
ISA / SE	13 July 1998	SE 98/00826	SE

Box No. VIII CHECK LIST; LANGUAGE OF FILING

This international application contains the following **number of sheets**:

request	:	4 ✓
description (excluding sequence listing part)	:	27 ✓
claims	:	4 ✓
abstract	:	1 ✓
drawings	:	5 ✓
sequence listing part of description	:	
Total number of sheets	:	31 35

This international application is **accompanied by** the item(s) marked below:

1. ☐ fee calculation sheet
2. ☒ separate signed power of attorney
3. ☒ copy of general power of attorney; reference number, if any: 243
4. ☐ statement explaining lack of signature
5. ☐ priority document(s) identified in Box No. VI as item(s):
6. ☐ translation of international application into (language):
7. ☐ separate indications concerning deposited microorganism or other biological material
8. ☐ nucleotide and/or amino acid sequence listing in computer readable form
9. ☒ other *(specify)*: ITS-Report

Figure of the drawings which should accompany the abstract: 1

Language of filing of the international application: Swedish

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

Nobel Biocare AB (publ)

[signature]

/ Gunnar Olsson / AGENT

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1. Date of actual receipt of the purported international application:	06 -07- 1999	2. Drawings: ☒ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:		
4. Date of timely receipt of the required corrections under PCT Article 11(2):		
5. International Searching Authority (if two or more are competent): ISA / SE	6. ☐ Transmittal of search copy delayed until search fee is paid.	

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Date of receipt of the record copy by the International Bureau: 09 AUGUST 1999 — 09. 08. 99

See Notes to the request form

Fig.1

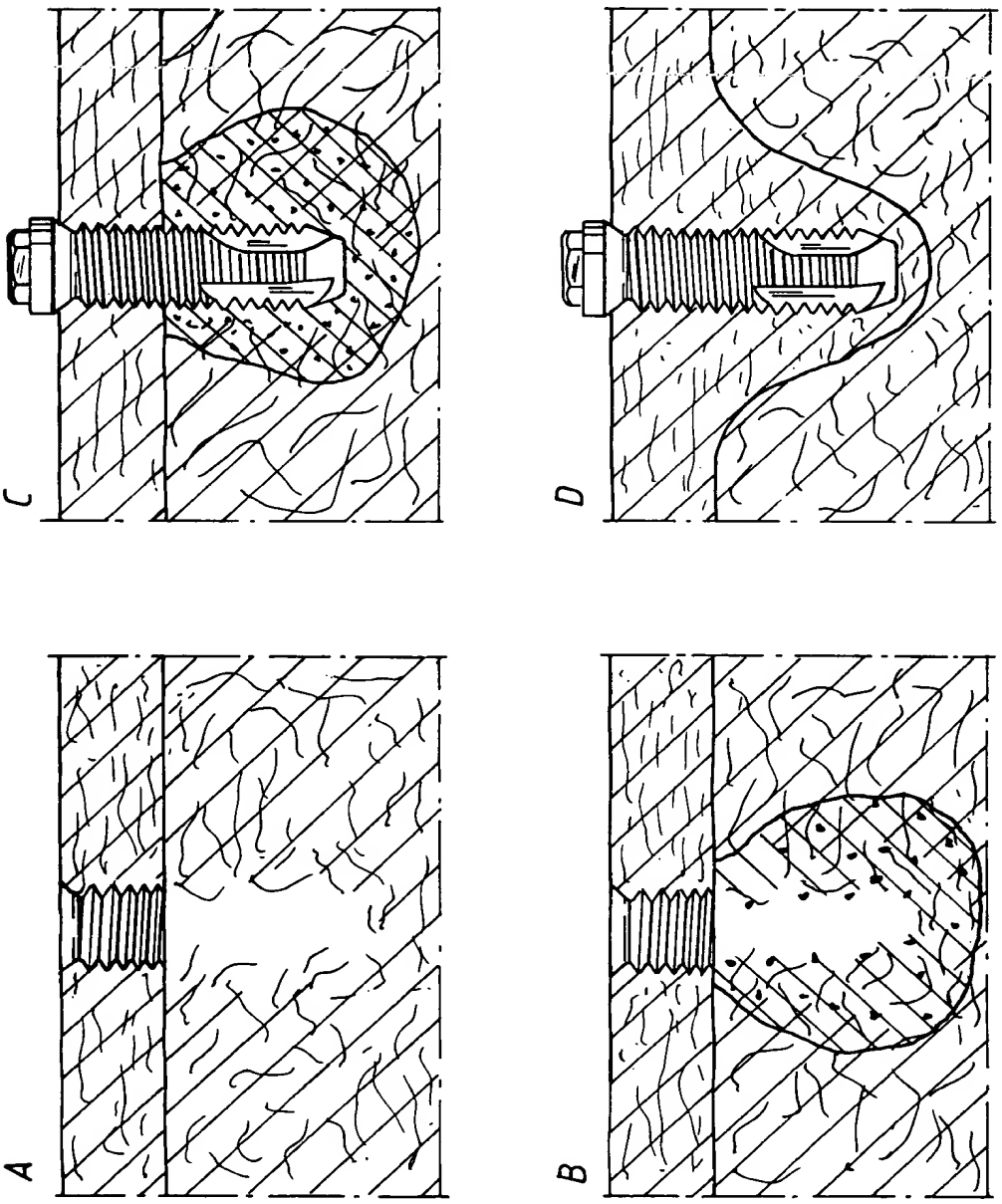
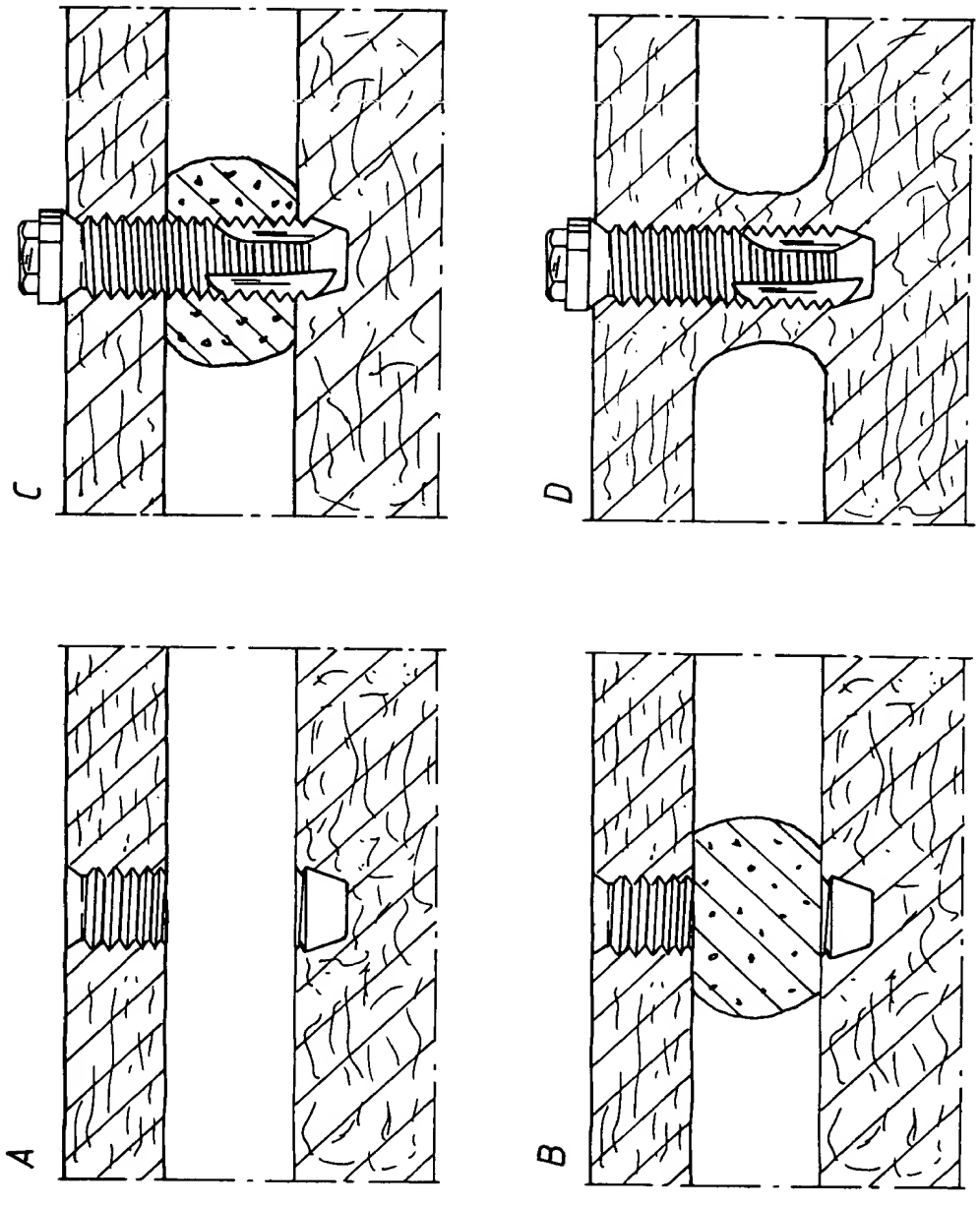


Fig. 2



08 -09- 1999

Fig. 3

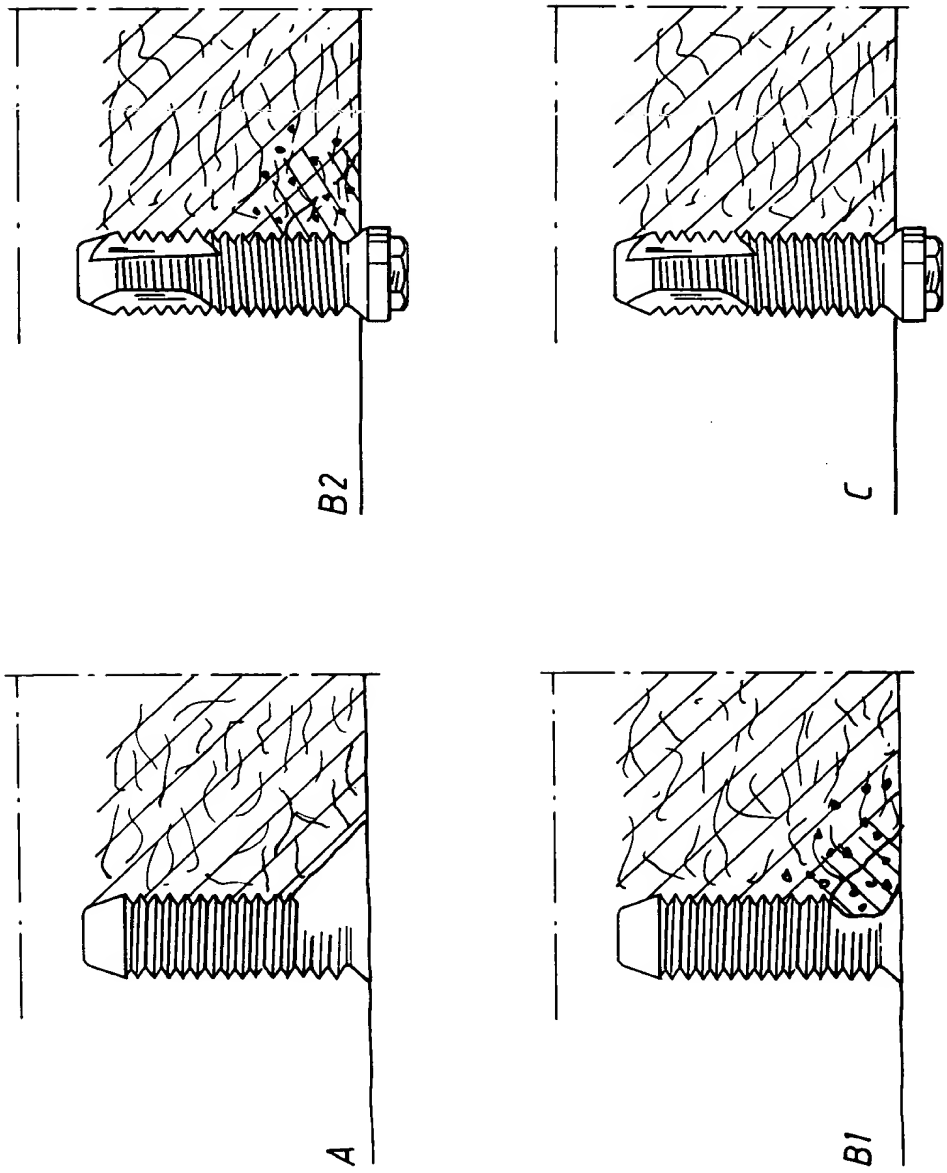


Fig. 4

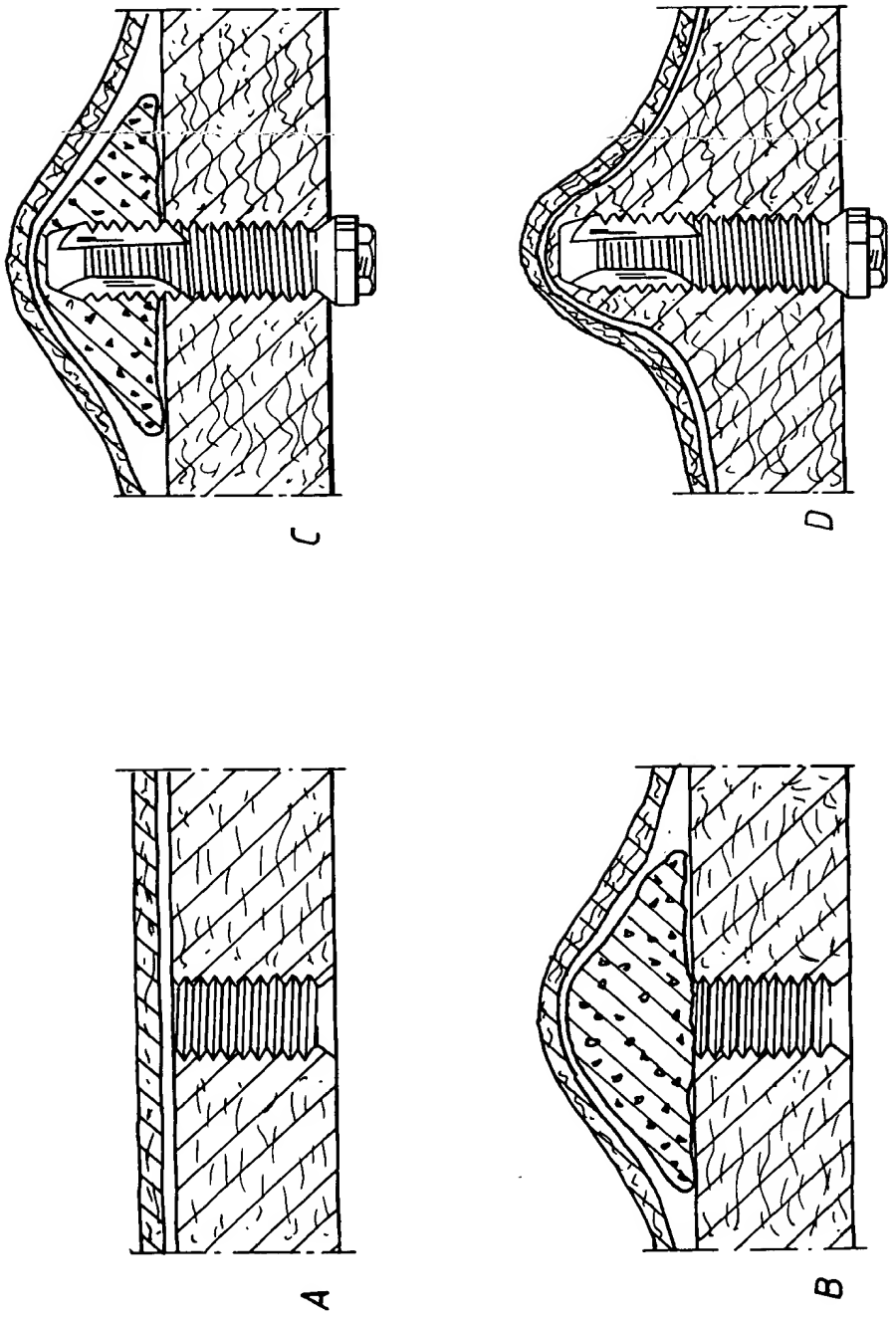
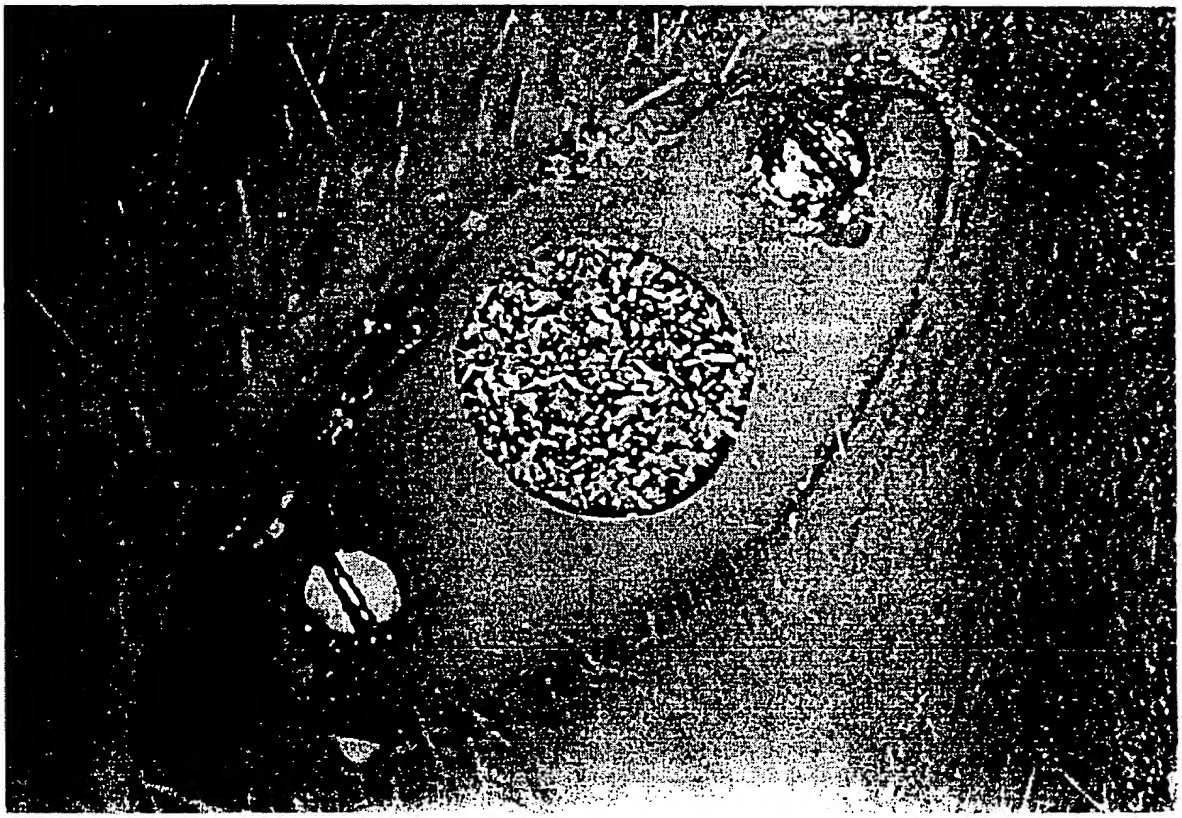


Fig. 5



Medel för benrekonstruktion

Uppfinningen avser ett medel för benrekonstruktion i kroppen hos människa eller djur i anslutning till en befintlig struktur, ett benimplantat eller annan protetisk konstruktion samt en metod för rekonstruktion av ben. Benrekonstruktionsmedlet utgöres av en lätt hanterbar och kontrollerbar beredning (komposition) avsedd att appliceras på det ställe där benet behöver ersättas, förstärkas eller byggas upp, speciellt i anslutning till ett benimplantat eller annan protetisk konstruktion där tillräcklig benvolym saknas, eller där benets kvalitet är för dålig för att medge lastbärande funktion, exempelvis permanent förankring av implantat.

Med benimplantat menas i det här sammanhanget exempelvis ett skruvformat, benförankrat implantat av titan eller titanlegering, s.k. fixtur, men innefattar även andra typer av implantat avsedda att installeras i benvävnad, inklusive ben från människa, speciellt partikulerat ben, men även i kombination med större kortikala och/eller spongiösa bentransplantat.

Bakgrund

Olika typer av bengrafter har använts mer eller mindre framgångsrikt för att ersätta förlorad benvävnad och förbättra läkning av bendefekter i syfte att rekonstruera funktion med och utan förankrade implantat. Vid autogen transplantation, vanligtvis från patientens egen höftkam, är det bland annat mängden ben och graden av resorption som påverkar behandlingsresultatet. Autolog bengrafting kräver vanligtvis mer än ett operationstillfälle för att uppnå ett tillfredsställande resultat och medför kraftiga postoperativa besvär för patienten. Vid homolog bengrafting används exempelvis deminerali-

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serat benmatrix från en s k benbank. Vävnader och struktur som gått förlorad p g a sjukdom eller skada kan idag till viss del ersättas av protetiska konstruktioner vilka mekaniskt förankras i skelettet. Höftproteser, konstgjorda knäleder och dentala implantat är exempel på hur förlorad vävnad, struktur och funktion kan ersättas med den här typen av konstruktioner.

Ersättning av förlorade tänder genom placering av dentala implantat i skelettet (käkarna) har hög lyckandefrekvens förutsatt att det finns tillräcklig mängd ben av god kvalitet i nära angränsning till och runt om implantatet. Efter en individuellt avpassad läkningstid (3 månader - 2 år) kan i de flesta fall protetiska konstruktioner förankras till de osseointegrerade (beninläkta) implantaten. En del patienter har p g a flerårig avsaknad av tänder fått sämre förutsättningar än andra att erhålla behandling med hjälp av benförankrade implantat. Det är främst områden i överkäken som drabbats kraftigast av bennedbrytning då tänderna förloras p g a anatomiska betingelser, men även områden långt bak i underkäken kan uppvisa undermåliga egenskaper för implantatbehandling.

Generella sjukdomstillstånd som t ex osteoporos och lokala defekter och avsaknad av ben på grund av t ex akuta skador, kongenitala defekter, kroniska infektioner eller lokala biologiska processer såsom cystor och tumörer i käkarna påverkar i de flesta fall och kan t o m omöjliggöra behandling med implantat om inte ben tillförs eller ersätts på något sätt för att öka mängden ben lokalt runt implantaten och därmed den initiala stabiliteten.

För de patienter som behandlas med implantat och benersättningmaterial är det viktigt att reducera inläkningstiden och garantera en högre lyckandefre-

kvens efter installationen än vad vi har möjlighet att göra idag. I decennier har försök gjorts att ersätta ben med organiska och syntetiska material från olika källor, se review, Smiler et al (1992)[1] inkluderande resorberbara och icke-resorberbara polymerer, bioaktivt glas, kalciumfosfatföreningar, kalciumkarbonater och naturligt förekommande material såsom ko-ben och korall. Att transplantera ben från patienten själv, s k autograft, är som beskrivet ovan ett alternativ, men ett relativt stort ingrepp som kräver specialistkompetens, sjukhusvård, en förlängd inläkningsperiod av transplantatet på minst sex månader samt extra besvär och smärta för patienten. Demineraliserat frystorkat ben från en s k benbank och spongiöst ben med mineral från ko-ben, innebär också en förlängd inläkningsperiod, risk för immunologiska reaktioner och infektiösa tillstånd, men även ett risktagande ur andra aspekter inkluderande lyckandefrekvensen.

Det finns således ett stort behov hos både specialister och icke-specialister att kunna applicera ett lättillgängligt, säkert medel för benrekonstruktion i samband med implantatbehandling hos de patienter som har otillräcklig volym ben och/eller för dålig benkvalitet.

Ett ändamål med denna uppfinning är att åstadkomma ett medel för benrekonstruktion som möjliggör implantatbehandling för patienter i olika situationer inom framför allt områden som annars ej kan behandlas och/eller har sämre prognos.

Kalciumfosfatföreningar är s k biokompatibla material, d v s material som är milt reaktiva med omgivningen, d v s främjar reparation och inläkning av t ex ett implantat, se Jarcho[2] och Lemons[3]. Den vanligaste formen av kalciumfosfatförening som används för att stimulera benbildning är hydroxylapa-

tit (HA), $Ca_{10}(PO_4)_6(OH)_2$, men även andra föreningar innehållande kalcium- och fosfatjoner förekommer och som liknar de oorganiska beståndsdelarna i skelett och emalj, se Daculsi et al (1997)[4]. Stökiometriskt HA, $Ca_{10}(PO_4)_6(OH)_2$, med Ca:P kvot på 1.67 hittas sällan *in vivo*. Kalcium är till viss del utbytt och ersatt av andra joner, såsom magnesium, natrium, aluminium, strontium, karbonat, fluor och klor, beroende på bl.a. individens ålder, föda, kön etc. HA kan förekomma i en keramisk och i en icke-keramisk form där graden av kristallinitet kan varieras beroende på under vilken temperatur kalciumfosfatföreningen framställs, Ricci et al (1992)[5].

Kalciumfosfatföreningar såsom hydroxylapatit finns kommersiellt tillgängligt och tillverkas av många olika företag; Impladent Ltd., Holliswood, New york, USA, Asahi Optical Co., Ltd., Tokyo, Japan, Interpore Int. och Irvine, California, USA. Materialen tillverkas med olika egenskaper såsom storlek på granulerna/partiklarna, grad av resorberbarhet och kemisk sammansättning.

Partiklarna/granulerna som resorberas gör det långsamt efter applikation i benvävnaden. Det anses att granulerna från början fysiskt upptar plats i defekten och därmed tillåter en påskyndad inläkningsprocess jämfört med en tom defekt. Under tiden det nya benet bildas, mineraliseras och remodelleras, resorberas granulerna långsamt ca 3-8 månader beroende på patientrelaterade faktorer inkluderande defektstorlek och patientålder.

Det finns experimentella studier, Hürzeler et al (1995)[6] och Wetzel et al (1995)[7] och kliniska studier, Smiler et al (1992)[1], Ricci et al (1992)[5], Judy (1986)[8] Wagner (1989)[9], och Corsair (1990)[10], som visar på en viss effekt av resorberbara granuler (Osteogen®) utblandade med t ex patientblod,

koksalt eller främst i kombination med demineraliserat frys-torkat ben. Möjligheten att göra en utfyllnad av sinus maxillaris, (överkäkens bihåla), för att öka möjligheterna till implantatförankring på hund, visar att resorberbara HA-granuler fungerar väl och produkten är lämplig att använda för att stimulera benbildning runt dentala implantat, Wetzel et al. (1995)[7]. Dock blandas granulerna med patientblod eller koksalt vilket gör produkten svår att hantera. En stor nackdel med denna beredningsform/teknik är att det är tekniskt svårt att föra granulmassan till defekt/kavitetområdet. Efter att granulerna applicerats är det stor risk att blod och andra kroppsvätskor från sårområdet späder ut och transporterar iväg materialet från applikationsområdet. En annan nackdel med en okontrollerad blandning av t ex koksalt eller patientens blod är att risken för kontamination av preparatet ökar.

Alper et al (1989)[11] har försökt att lösa hanteringsproblemen. Dock visar denna referens att generellt stimulerar inte en formbar hydroxylapatitberedning baserad på fosfolipider och stearinsyra bildning av nytt ben eller bentillväxt utan istället pekar resultaten på en reducering av benbildningen.

Lipider kan indelas i olika klasser. Triglycerider är den mest förekommande klassen av lipider och är en viktig energidepot i celler. Triglycerider antingen byggs upp eller bryts ned i kroppen via diglycerider från eller till monoglycerider och fettsyror. I kroppen förekommer också olika typer av membranlipider, exempelvis fosfatidylkolin, fosfatidyletanolamin, sfingomyelin, kolesterol, mono- och digalaktosyldiacylglycerol.

Fosfolipider kan framställas helt syntetiskt, men också renas från biologiska råvaror såsom växter

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eller djur. Exempel på råvaror är äggula, vegetabiliska oljor såsom sojabönsolja, rapsolja osv.

Att föredra är också att beredningen innehåller antioxidantia valda enligt kända principer eller naturligt förekommande. Ett exempel på en fördelaktig antioxidantia i det här fallet kan vara tokoferol. Liposomer består av ett sfäriskt skal av amfifila lipider som innesluter en vattenfas. Dessa lipidvesiklars potential som bärare för läkemedel har studerats och beskrivits i ett flertal artiklar. Huang et al[12] har föreslagit att negativt laddade liposomer kan påverka mineraliseringsprocessen av nybildat ben. Detta koncept testades i en defektmodell på minigris dock utan effekt. Benbildning mellan obelagda och liposombelagda kalciumfosfatföreningar jämfördes och de obelagda kalciumfosfatföreningarna var omgivna av mer ben och mer moget ben än de liposombelagda. I en experimentell studie av Raggio et al (1986)[13] visar författarna att komplexa sura lipider påverkar hydroxylapatit-mineral-utfällning i en fysiologisk miljö.

På senare tid har en del forskning fokuserat på tillämpningen av att introducera exogena molekyler in i celler med hjälp av lipidkomplex. Dessa nya lipider har en viktig klinisk tillämpning för "drug-delivery" och genterapi. Eftersom lipiderna kan skräddarsys med olika fysiska egenskaper kan tillämpningen varieras, Ashley et al (1996)[14] och Barber et al (1996)[15].

Olika system baserade på kalciumfosfatgranuler och lipid bärare finns beskrivna i litteraturen, se exempelvis EP 0429419 som visar ett system där kalciumfosfat, speciellt hydroxylapatit används som benersättningsmaterial. Härvid används en monoglycerid-baserad bärare vilket kan innebära en nackdel vid implantatbehandling eftersom preliminära studi-

er visar att kapselbildning kan förekomma vilket i sin tur kan påverka implantatinläkning negativt.

Olika system för frisättning av läkemedel som innehåller biokeramiska granuler och lipid finns också beskrivna i litteraturen sen tidigare, se exempelvis JP 2,631,890. Som exempel på olika bärare för droger och molekyler som ska frisättas i benvävnad kan nämnas kollagen, lipider, polymerer (t ex PLA/PGA och hyaluronsyra) och keramer.

Åtskilliga studier visar att mineraldeponering i brosk som håller på att förkalkas först hittas i phophatidylserine- och alkaliskt phosphatase-innehållande vesiklar och att den endokondrala förkalkningsprocessen vid tillväxtplattan i epifysen kan vara medierad av dessa. Matrixvesiklar och de negativt laddade fosfolipider som finns i dem förefaller vara involverade i den initiala bildningen av kalciumhydroxylapatitkristaller via interaktionen mellan kalcium och fosfatjoner med phosphatidylserine i bildandet av phospholipid:kalcium:fosfatjon-komplex, Boyan et al (1989)[16].

Det är främst de systemiskt blodkalciumreglerande hormonerna som på olika sätt styr benceller och därmed håller kroppens benmassa i balans. På senare tid finns det många studier som indikerar att vissa biopolymerer av polypeptidtyp producerade av benceller själva och/eller blodceller från benmärg eller vid inflammation efter t ex trauma, har en viktig och sannolikt en mer direkt betydelse för aktivering av de enskilda cellerna i samband med benbildningsprocessen.

Benbildning och benresorption är kopplade till varandra. Systemiskt och lokalt producerade faktorer reglerar processerna. Många av tillväxtfaktorerna kan ha olika effekt på olika celler. T.ex. kan PTH

och vitamin D stimulera benresorption och remodellering via de benbildande cellerna, se Nijweide et al. (1986)[17]. De benbildande cellerna kan å andra sidan stimuleras av TGF-beta frisatt från matrix under benresorptionsprocessen, se Pfeilschifter et al. (1990)[18].

De tillväxtfaktorer och cytokiner som produceras av benceller kan ha en autokrin eller parakrin effekt. Exempel på dessa är: TGF, IGF-I och IGF-II, Beta2 Mikroglobulin, PDGF och CSFs. Trombocytderiverade faktorer såsom TGF, PDGF och EGF, men även interleukiner, TNFs och Interferon-gamma är faktorer av hematologiskt ursprung som har effekt på de benbildande cellerna. Tillväxtfaktorer som finns lagrade i benmatrisen är den allra största reservoaren för tillväxtfaktoraktivitet. De faktorer som finns lagrade är som nämnts ovan TGF, IGF-I och IGF-II, Beta2 Mikroglobulin, PDGF, men även FGF. Bone morphogenetic proteins, BMP, och osteogenin tillhör TGF-familjen. Vanligtvis brukar BMP kombineras med urkalkat benmatrix och kollagen, se Sampath och Reddi (1981)[19] och Saito et al. (1994)[20]. Kuboki et al. (1995)[21] har visat att BMP inducerar bara ben bättre om HA-bäraren består av partiklar som är porösa jämfört med icke-porösa.

I en nyligen publicerad experimentell studie av Urist et al (1997)[22] undersöks olika system för administration av tillväxtfaktorn BMP-2 och dess effekt på benbildning. Författarna föreslår att lipider som extraherats från ben kan fungera väl som bärare för benstimulerande peptider i benbildningsprocessen.

Andra molekyler eller joner som kan binda starkt till kristallytor är exempelvis bisphosphonater som kan påverka osteoblaster och därmed upplösning av

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kalciumfosfatföreningar i skelettet, se Ebrahimpour et al. (1995)[23] .

Enligt uppfinningen utgöres medlet för benrekonstruktion av en blandning av resorberbara kalciumfosfatgranuler och/eller en bärare av biopolymer eller lipidtyp där lipiden innehåller en förestrad fettsyra vald från gruppen triglycerider, diglycerider eller fosfolipider eller kombinationer därav. Uppfinningen avser att övervinna de svårigheter som beskrivits ovan och utgöra en beredning som lätt och på ett upprepbart sätt kan användas i samband med benimplantat. Mer specifikt menas att materialet enligt uppfinningen ska motstå utspädning och transportering bort från applikationsområdet. En sådan blandning kan ges "rätt" konsistens beroende på applikationsform, kan exempelvis göras formbar, och är lätt att hantera, kontrollera och applicera.

Ett önskvärt viktförhållande mellan kalciumfosfat och fosfolipid är i storleksordningen 70:15 till 60:40. Ett önskvärt viktförhållande mellan fosfolipid och vatten eller andra vattenbaserade vätskor är i storleksordningen 1:2 till 10:1, helst 3:2 till 4:1.

Med hänvisning till den vattenbaserade vätskan som används för att göra beredningen formbar föredras rent vatten, isoton saltlösning eller en farmaceutisk accepterbar lösning. Vid vissa fall då beredningen färdigställs in situ, kan kroppsvätskor inkluderande blod användas.

I det följande skall uppfinningen närmare beskrivas i samband med några olika applikationer av benimplantat där den omgivande benvävnaden behöver förstärkas och/eller byggas upp, varvid

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figur 1 visar ett fall där benet bildar ett alltför glest, lastbärande nätverk,

figur 2 visar ett fall där den laterala benvolymen är otillräcklig,

figur 3 visar ett fall med angulära och/eller för smala benkristor,

figur 4 visar ett fall där den vertikala benhöjden är otillräcklig, och

figur 5 visar en bild på applicerad beredning i kaninben.

Som nämndes inledningsvis utgörs medlet för benrekonstruktion av en blandning av resorberbara kalciumfosfatgranuler och en bärare av biopolymer eller lipid typ. För att kunna appliceras i anslutning till ett benimplantat och hållas kvar på i applikationsområdet är det viktigt att blandningen är formbar och har rätt konsistens. Om partiklarna transporteras bort från applikationsområdet finns möjligheten att dom skulle kunna orsaka irritation eller komplikation på andra ställen i kroppen.

Kalciumfosfatgranulerna bör ha en Ca/P kvot som ligger mellan 1 och 2. Granulerna bör ha en medeldiameter på 0.05 - 5 mm och en mikro/makroporositet på 0-80%.

I en studie av Neo et al (1992)[24] studerades gränszonen mellan bioaktiva keramer och ben med hjälp av svepelektronmikroskopi och transmissionselektronmikroskopi. Kalciumfosfatgranuler med en medeldiameter på 0.1 till 0.3 mm studerades och karaktäriserades med avseende på resorberbarheten. Efter 8 veckor var de icke-resorberbara granulerna förbund-

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na med ben genom ett tunt Ca-P rikt lager bestående av fina apatit-kristaller, dock annorlunda än de som finns i ben vad avser form, storlek och orientering. De resorberbara granulerna däremot hade direktkontakt med benet. Granulernas yta var råare på grund av degradering och analyser visade att ben växte in i de minsta ytoregelbundenheterna. I en annan studie av Kitsugi et al[25] jämfördes fyra olika typer av kalciumfosfatkeramer. Ca/P-kvoten var i det fallet 1, 1.5 och 1.66 och partiklarnas (granulernas) storlek varierade mellan 0.15 och 0.3 mm. Transmissionselektonmikroskopiska observationer visade att Ca/P-kvoten ej påverkade förbindelsen och kontakten mellan partiklarna och det omgivande benet.

Enligt uppfinningen fordras en bärare för de resorberbara kalciumfosfatgranulerna, vilken kan utgöras av en biopolymer eller lipid som innehåller estrar av fettsyror, såsom triglycerid, diglycerid, eller fosfolipider eller kombinationer därav, exempelvis någon av de lipider som beskrivs i WO 95/34287.

Företrädesvis är kalciumfosfatgranulerna fördelade i en lamellär, flytande, kristallin fas som innehåller åtminstone en fosfolipid och bildas antingen i kroppen eller dessförinnan.

Exempel 1

Följande exempel beskriver ett test på hanterbarheten hos en HA-granul/lipidblandning varvid en fosfolipid (Epikuron 200 från Lucas Meyer) blandats med HA-granuler (I detta fall Apaceram från Pentax) och etanol, serie A prov 1-8. Dessa prover har sedan frystorkats till konstant vikt. Proverna har en sammansättning efter frystorkningen som framgår av nedanstående tabell.

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I serie B har prover 9-17 blandats enligt nedanstående tabell, varefter formbarheten har bedömts. Proverna gjordes genom att 0.9 vikts-% NaCl i vatten (fysiologisk koksaltlösning) vägdes in och blandades med fosfolipid (PC) varefter hydroxyapatitgranuler tillsattes. Alla prov i denna serie innehåller 70 vikt% HA och mängden granuler har hållits konstant medan förhållandet fosfolipid till vatten varierats. De prover som endast innehåller två komponenter PC och granuler, samt vatten och granuler, hade inte en tillfredsställande hanterbarhet.

Serie A, prov 1-8

PROV NR	PC %	HA %	KONSISTENS
1	10.1	89.9	smulig
2	15.1	84.9	smulig
3	20.1	79.9	smulig
4	25.0	75.0	något formbar
5	30.1	69.9	formbar
6	35.1	64.9	formbar
7	40.0	60.0	formbar
8	45.0	55.0	formbar

Serie B, prov 9-17

PROV NR	PC %	0.9% NACL ISG	HA %	KONSISTENS
9	3.2	27.0	69.9	smulig
10	6.0	24.0	70.0	smulig
11	9.2	20.9	69.9	smulig, något formbar
12	12.0	18.1	69.9	smulig, något formbar
13	15.0	15.2	69.9	något formbar
14	18.0	12.0	70.0	formbar
15	21.1	9.1	69.8	formbar
16	24.1	6.1	69.8	formbar
17	27.0	3.0	70.0	smulig, något formbar

Viktsförhållandet mellan kalciumfosfatkomponenten och lipiden samt vatteninblandning bestäms av kravet på att beredningen skall vara lätt hanterbar och formbar. Företrädesvis bör viktsförhållandet mellan kalciumfosfat och fosfolipid ligga inom intervallet 70:15 till 60:40. Viktsförhållandet fosfolipid till vatten eller annan vattenbaserad vätska bör ligga inom intervallet 1:2 till 10:1, företrädesvis 3:2 till 4:1.

Exempel 2

Formbara beredningar tillverkades genom att blanda 0.21 gram fosfolipid (1,2-dioleyl-sn-glycero-3-phosphocholine, Avanti Polar Lipids, Inc.) med 0.12 gram 0.9% fysiologiskt koksalt. Efter en timme tillsattes 0.71 gram hydroxyapatit granuler (OsteoGen® (HA Resorb)®, Impladent Ltd.). Beredningen blandades till en formbar konsistens och var lätt att packa i en applikator.

Beredningen utvärderades med avseende på hanterbarhet och applicerbarhet i en defektmodell där cirkulära defekter med diam. 4 mm skapats i underbenet (tibia) på vuxna New Zealand White-kaniner. Utvärderingen visar att beredningen var hanterbar och lätt att applicera i defektområdet med en modifierad spruta. Beredningen hölls ihop och påverkades ej av det relativt kraftiga blodflödet från sårområdet. I figur 5 visas en bild av den applicerade beredningen insatt i kaninbenet. Utvärdering görs nu med avseende på mängd nybildat ben (histomorfometriskt) i defektområdet och resultatet kommer att jämföras parvist med en obehandlad defekt skapad på identiskt sätt.

Istället för en lipid av det slag som beskrivits ovan kan bäraren utgöras av en biopolymer,

enproteoglykan, en glykosaminoglykan, exempelvis hyaluronsyra. Hyaluronsyra är en anjonisk polysackarid sammansatt av repeterade disacakaridenheter av beta-1-4-glukoronat-beta-1-3-N-acetyl-glukosamin och är en del av den extracellulära matrisen. Hyaluronsyra fungerar som smörjmedel i leder, finns i stora kvantiteter i bindväv samt är rikligt förekommande i ögat. I en studie där det undersökts om fagocyterbara partiklar av hydroxyapatit har en skadlig effekt på benbildningen, har natriumhyaluron använts som bärarlösning för hydroxyapatit, se Wang J-S, et al.(1994)[26].

Följande formulering av hyaluronsyra och hydroxyapatit kan användas som benersättningsmedel enligt uppfinningen. En frystorkad blandning av natriumhyaluron (Healon®) och hydroxyapatit (65:35%w/w) sväller i närvaro av vatten och är därmed lätt att forma och hantera. Den frystorkade blandningen av natriumhyaluron och hydroxyapatit packas lätt i en applikator och/eller modifierad spruta vilken rehydreras med vatten, vattenlösning eller kroppsvätska i nära anslutning till appliceringen för att bilda en formbar hydroxyapatit-formulering för benrekonstruktion.

Benrekonstruktionsmedlet enligt uppfinningen eller granulerna i sig kan med fördel kombineras med någon i och för sig känd cellstimulerande substans, t.ex. tillväxtfaktorer såsom BMP och tillhörande TGF-beta-familjen. Benstimulerande substanser och molekyler överför signaler och påverkar celler och cellulära aktiviteter i ben. Proteiner och polypeptider är exempel på substanser som visat sig på olika sätt kunna ha en benstimulerande effekt, främst lokal. Förutom att beredningen kan blandas med benimplantat från patienten själv kan kalciumfosfatgranuler tillföras innehållande benstimulerande substanser eller delar därav.

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Beroende på de beninducerande eller vävnadsfrämjande faktorernas egenskaper så existerar vissa av dem i en aktiv respektive inaktiv form och behöver kombineras med olika typer av bärare för att inducera ben och brosk *in vivo*. Förklaringen till detta var först att tillväxtfaktorn diffunderade iväg för snabbt och inte hölls på plats tillräckligt länge för att uppnå effekt. Senare studier har visat att bäraren fungerar även som stöd och underlag för celler att fästa till och differentiera på eftersom benbildningen endast kan ske på en yta eller substrat, se Kuboki et al. (1995)[21]. Vanliga exempel på bärare för BMP är urkalkat benmatrix och kollagen, se Sampath och Reddi (1981)[19] och Saito et al. (1994)[20]. Kuboki et al. (1995)[21] har visat att BMP inducerar bara ben om HA-bäraren består av partiklar som är porösa. Tsuruga et al. (1997)[27] har teorier om att celldifferentiering och benbildning är starkt beroende av bäraren i egenskap av substratyta och mikromiljö. Enligt uppfinningen föreslås således att den formbara kalciumfosfatgranul-lipidblandningen får utgöra bärare för t.ex. tillväxtfaktorer.

I det följande visas i samband med figurerna 1-4, några exempel på situationer där uppfinningen kan tillämpas:

Figur 1.

A. Efter uppborrning (och förgängning) av benet B. appliceras beredningen i det skapade hålet med hjälp av en spruta eller ett insereringsinstrument. Beredningen trycks ut mot kavitetsväggarna och på grund av att benarkitekturen utgörs av inget eller ett glest nätverk möjliggörs penetration av beredningen perifert ut i vävnaden. C. Ett skruvformat

benimplantat (fixtur) installeras enligt en standardprocedur och efter inläkning har benet runt fixturen remodellerats och antagit ett mer lamellärt strukturellt utseende och har därmed också möjlighet att uppta belastning på ett mer fördelaktigt sätt.

Figur 2

A.Efter uppborrning (och förgängning) B. appliceras beredningen i det skapade hålet med hjälp av t ex en spruta eller liknande insereringsinstrument. Beredningen appliceras i defekten och på grund av beredningens konsistens stannar granulerna kvar i applikationsområdet och möjliggör därmed nybildning av ben i defektområdet. C. Fixturen kan installeras enligt en standardprocedur direkt genom bikortikal förankring, D. men även efter en kortare eller längre inläkningsperiod då benet i området omvandlats och antagit ett mer lamellärt strukturellt och kortikalt utseende.

Figur 3

A. Efter uppborrning (och förgängning) B1. appliceras beredningen före fixturen installeras eller B2 efter att fixturen installerats i fall med otillräcklig vertikal och horisontell benvolym t ex angulära defekter och/eller för smala benkristor. Dessa två situationer är exempel på situationer då en del av fixturen initialt ej har tillfredsställande kontakt med omgivande benvävnad. C. Efter inläkning har läkningsförloppet återskapat den normala benarkitekturen i området.

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Figur 4

Efter tandlöshet i överkäken har i många fall sinushålan expanderat samtidigt som benkristan resorberats. Detta medför att den vertikala höjden som krävs för förankring av fixturer är otillräcklig.

Alt. I A. Efter uppborrning (och förgängning) B. appliceras beredningen med en modifierad spruta eller annat instrument under sinusslemhinnan. C. Fixturen installeras och efter obelastad inläkning medför beredningen att nytt ben bildas i anslutning till och i kontakt med fixturen.

Alt. II Vid en så kallad "sinus-lift" kan beredningen även appliceras lateralt ifrån, d v s kommunikation med sinushålan sker genom en lateralt skapad defekt varefter sinusslemhinnan lyftes och delar av botten av sinushålan kan fyllas med beredningen.

Figur 5

Bilden visar en beredning enligt exempel 2 ovan applicerad i ett kaninben, så som redan beskrivits ovan.

Referenser

1. Smiler D. G., Johnson P. W., Lozada J. L., Misch C., Rosenlicht J. L., Tatum O. H. And Wagner J. R. (1992), Sinus lift grafts and endosseous implants. Treatment of the atrophic posterior maxilla. *Dent Clin North Am*, Jan 1, 36 (1), 151-186.

2. Jarcho M. (1981), Calcium phosphate ceramics as hard tissue prosthetics. *Clin Orthop and Rel Res*, 157, 259-278.

3. Lemons J. E. (1996), Ceramics: Past, present and future. *Bone* 19 (1) Suppl., 121S-128S.

4. Daculsi G., Bouler J.-M., and LeGeros R. Z. (1997), Adaptive crystal formation in normal and pathological calcifications in synthetic calcium phosphate and related biomaterials. *International Review of Cytology* 172, 129-191.

5. Ricci J. L., Blumenthal N. C., Spivak J. M. and Alexander H., (1992), *J Oral Maxillofac Surg* 50, 969-978.

6. Hürzeler M. B., Quiñones C. R., Morrison E. C., Caffesse R. G., (1995) Treatment of periimplantitis using guided bone regeneration and bone grafts, alone or in combination, in beagle dogs. Part 1: Clinical findings and histologic observations, *Int J Oral Maxillofac Implants*, 10, 474-484.

7. Wetzel A. C., Stich H., and Caffesse R. G., (1995), Bone apposition onto oral implants in the sinus area filled with different grafting materials. A histological study in beagle dogs. *Clin Oral Impl Res*, 6, 155-163.

8. Judy K. W. M., (1986) Oral Implantology Case Reports, *The New York State Dental Journal*, 52 (10), 24-26.

9. Wagner J. R., (1989) A clinical and histological case study using resorbable hydroxylapap-

tite for the repair of osseous defects prior to endosseous implant surgery. *J Oral Implant*, XV, 3, 186-192.

10. Corsair A., (1990) A clinical evaluation of resorbable hydroxylapatite for the repair of human intra-osseous defects, *J Oral Implant*, XVI, 2, 125-128.

11. Alper G., Bernick S., Yazdi M., and Nimni ME. (1989). Osteogenesis in bone defects in rats; the effects of hydroxyapatite and demineralized bone matrix. *Am J Med Sci. Dec;* 298(6): 371-376.

12. Huang J-S., Liu K-M., Chen C-C., Ho K-Y., Wu Y-M., Wang C-C., Cheng Y-M., Ko W-L., Liu C-S., Ho Y-P., Wang Y-P. And Hong K. (1997). Liposomescoated hydroxyapatite and tricalciumphosphate implanted in the mandibular bony defect of miniture swine. *Kaohsiung J Med Sci* 13; 213-228.

13. Raggio C. L., Boyan B. D., and Boskey A. L. (1986), In vivo hydroxyapatite formation induced by lipids, *J Bone Miner Res*, Oct; 1(5), 409-415.

14. Ashley G. W., Shida M. M., Qiu R., Lahiri M. K., Levisay P. C., Jones R. D., Baker K. A., and Macdonald R. C. (1996), Phosphatidylcholinium compounds: A new class of cationic phospholipids with transfection activity and unusual physical properties. (abstract) *Biophysical J.* 70: A88.

15. Barber K., Mala R. R., Lambert M. P., Qiu R., MacDonald R. C. and Klein W. L. (1996), Delivery of membrane-impermeant fluorescent probes into living neural cell populations by lipotransfer. *Neurosci letter*, 207, 17-20.

16. Boyan B. D., Schwartz Z., Swain L. D., and Kahre A. (1989), Role of lipids in calcification of cartilage, *Anat Rec*, 224(2), 211-219.

17. Nijweide P.I., Burger E.H., Feyen I.H.M., (1986), Cells of bone; proliferation, differentiation and hormonal regulation. *Physiol. Rev.*, 66:855-886.

18. Pfeilschifter I., Wolf O., Nauman A., Minne H.W., Mundy G.R., Ziegler R., (1990). Chemotactic response of osteoblastlike cells to transforming growth factor beta. *J.Bone Min. Res*.5:825-830

19. Sampath T.K. and Reddi A.H., (1981). Dissociative extraction and reconstruction of extracellulär matrix components involved in local bone differentiation. *Proc. Natl. Acad. Sci.* USA 78, 7599-7603.

20. Saito A., Kato H. And Kuboki Y., (1994). A study of periodontal regenerative therapy using BMP in horizontal bone defects. *Jpn J Periodont.* 36, 810-822.

21. Kuboki Y., Saito T., Murata M., Takita H., Mizuno M., Inoue M., Nagai N. and Pool R. (1995). Two distinct BMP-carriers induce zonal chondrogenesis and membranous ossification, respectively; geometrical factors of matrices for cell-differentiation. *Connect. Tissue. Res.* 32, 219-226..

22. Urist M. R., Behnam K., Kerendi F., Raskin K., Nuygen T. D., Shamie A. N., and Malinin T. I. (1997), Lipids closely associated with bone morphogenetic protein (BMP) ... and induced heterotopic bone formation. With preliminary observations of deficiencies in lipid and osteoinduction in lathyrism in rats, *Connective Tissue Research* 36(1), 9-20.

23. Ebrahimpour A., and Francis M.D. (1995), Bisphosphonate therapy in acute and chronic bone loss: Physical chemical considerations in biophosphonate related therapies. *In: Bisphosphonate on Bones*. Eds. Bijvoet O:L:M, Fleisch H.A., Canfield R.E. and Russell R.G.G., Elsevier, Amsterdam.

24. Neo M., Kotani S., Fujita Y., Nakamura T., Yamamuro T., Bando Y., Ohtsuki C. och Kobuko T.

(1992) Differences in ceramic-bone interface between surface-active ceramics and resorbable ceramics; a study by scanning and transmission electron microscopy. *J Biomed Mater Res.*, Feb;26(2):255-267.

25. Kitsugi T., Yamamuro T., Nakamura T., and Oka M. (1995). Transmission electron microscopy observations at the interface of bone and four types of calcium phosphate ceramics with different calcium/phosphorus molar ratios. *Biomaterials* Sep;16(14):1101-1107.

26. Wang J-S, Goodman S., Aspenberg P. Bone formation in the presence of phagocytosable hydroxyapatite particles, *Clin. Orthop.* 304, 272-279, 1994.

27. Tsuruga E., Takita H., Itoh H., Wakisaka Y. And Kuboki Y. (1997). Pore size of porous hydroxyapatite as the cell-substratum controls PMP-induced osteogenesis. *J. Biochem.* 121, 317-324.

PATENTKRAV

1. Medel för benrekonstruktion i kroppen hos människa eller djur i anslutning till en befintlig struktur, ett benimplantat eller annan protetisk konstruktion varvid medlet är avsett att appliceras på det ställe i anslutning till exempelvis ett benimplantat eller annan protetisk konstruktion där tillräcklig benvolym saknas, eller där benets kvalitet är för dålig eller för att medge en lastbärande funktion k ä n n e t e c k n a t a v att medlet utgöres av en lätt hanterbar, kontrollerbar och nedbrytbar bärarberedning av kalciumfosfatgranuler och en biologisk organisk komponent av biopolymer och/eller lipid typ.

2. Medel enligt patentkrav 1 k ä n n e t e c k n a t a v att beredningen har en formbar konsistens.

3. Medel enligt patentkrav 2 k ä n n e t e c k n a t a v att beredningen gjorts formbar genom inblandning av vatten eller annan vattenbaserad vätska, exempelvis kroppsvätska.

4. Medel enligt patentkrav 2 k ä n n e t e c k n a t a v att lipiden utgöres av en blandning av förestrad glycerol och fosfolipid.

5. Medel enligt patentkrav 4 k ä n n e t e c k n a t a v att den förestrade glycerolen utgöres av di- och triglycerid.

6. Medel enligt patentkrav 4 k ä n n e t e c k n a t a v att den förestrade glycerolen är en diester.

7. Medel enligt patentkrav 4 k ä n n e t e c k -

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n a t a v att den förestrade glycerolen är en triester.

8. Medel enligt patentkrav 2 k ä n n e t e c k - n a t a v att lipiden utgörs av en blandning av fosfolipider.

9. Medel enligt patentkrav 8 k ä n ne t e c k - n a t a v att fosfolipiden är en sfingomyelin.

10. Medel enligt patentkrav 8 k ä n n e t c k - n a t a v att fosfolipiden är en fosfatidylkolin.

11. Medel enligt patentkrav 2 k ä n n e t e c k - n a t a v att lipiden är framställd från en växtolja eller äggula.

12. Medel enligt något av föregående patentkrav k ä n n e t e c k n a t a v att lipiden utgöres av åtminstone en fosfolipid och vatten eller annan vattenbaserad vätska som bärare.

13. Medel enligt patentkrav 12 k ä n n e t e c k- n a t a v att lipiden är i lamellär flytande kristallin fas.

14. Medel enligt patentkrav 12 k ä n n e t e c k - n a t a v att viktsförhållandet mellan lipid och vatten eller annan vattenbaserad vätska ligger inom storleksområdet 1:2 till 10:1, företrädesvis 3:2 till 4:1.

15. Medel enligt patentkrav 2 k ä n n e t e c k - n a t a v att biopolymeren innehåller en glykosaminoglykan, exempelvis hyaluronsyra.

16. Medel enligt patentkrav 15 k ä n n e t e c k - n a t a v att det utgöres av en friflytande blandning av natrium-hyaluron och kalciumfosfatgra-

nuler som kan packas och sedan rehydreras i samband med användning.

17. Medel enligt patentkrav 1 k ä n n e t e c k n a t a v att kalciumfosfatgranulerna har en Ca/P kvot som ligger mellan 1 och 2.

18. Medel enligt patentkrav 17 k ä n n e t e c k n a t a v att kalciumfosfaten innehåller hydroxyapatit av formen $Ca_{10}(PO_4)_6(OH)_2$.

19. Medel enligt patentkrav 17 k ä n n e t e c k n a t a v att kalciumfosfaten innehåller dikalcium-fosfat-dihydrat, octakalcium-fosfat, trikalciumfosfat och/eller hydroxyapatit.

20. Medel enligt patentkrav 17 k ä n n e t e c k n a t a v att kalciumfosfaten innehåller magnesium-, fluor-, eller karbonatjoner.

21. Medel enligt patentkrav 17 k ä n n e t e c k n a t a v att kalciumfosfatgranulerna har en diameter i storleksordningen 0.05 mm till 5 mm.

22. Medel enligt patentkrav 17 k ä n n e t e c k n a t a v att kalciumfosfatgranulerna har en porositet på 0-80%.

23. Medel enligt något av föregående patentkrav k ä n n e t e c k n a t a v att viktsförhållandet mellan kalciumfosfatgranulerna och lipiden ligger inom storleksområdet 70:15 till 60:40.

24. Medel enligt något av föregående patentkrav k ä n n e t e c k n a t a v att det innehåller vävnadsfrämjande faktorer och/eller faktorer som hämmar nedbrytning av vävnad, exempelvis en tillväxtfaktor, såsom BMP och TGF-beta eller delar därav.

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25. Medel enligt patentkrav 3, 16 och 24 k ä n n e t e c k n a t a v att den vävnadsfrämjande faktorn helt eller delvis tillsätts.

26. Metod för rekonstruktion av ben i kroppen hos människa eller djur i anslutning till en befintlig struktur, ett benimplantat eller annan protetisk konstruktion k ä n n e t e c k n a d a v att en lätt hanterbar och kontrollerbar beredning av resorberbara kalciumfosfatgranuler och en biologisk organisk bärare av biopolymer eller lipid typ appliceras på det ställe i kroppen i anslutning till exempelvis ett benimplantat eller annan protetisk konstruktion där tillräcklig benvolym saknas, eller där benets kvalitet är för dålig för att medge en lastbärande funktion.

27. Metod enligt patentkrav 26 k ä n n e t e c k n a d a v att beredningen appliceras i en skapad eller befintlig kavitet eller defekt i benet med hjälp av en spruta eller planinstrument.

28. Metod enligt patentkrav 26 k ä n n e t e c k n a d a v att beredningen appliceras i en för ett benimplantat skapad kavitet.

29. Metod enligt patentkrav 26 k ä n n e t e c k n a d a v att beredningen appliceras i en bendefekt, exempelvis en angulär defekt i anslutning till ett redan installerat benimplantat eller en för ett benimplantat skapad kavitet.

30. Metod enligt patentkrav 26 k ä n n e t e c k n a d a v att beredningen appliceras på benet under sinusslemhinnan i avsikt att öka den vertikala höjden hos benkristan.

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SAMMANDRAG

Uppfinningen avser ett medel för benrekonstruktion i kroppen hos människa eller djur i anslutning till en befintlig struktur, ett benimplantat eller annan protetisk konstruktion samt en metod för rekonstruktion av ben. Benrekonstruktiomsmedlet utgöres av en lätt hanterbar och kontrollerbar beredning (komposition) av resorberbara kalciumfosfatgranuler och en bärare av biopolymer eller lipid typ. Medlet är avsett att appliceras på det ställe där benet behöver ersättas, förstärkas eller byggas upp, speciellt i anslutning till ett benimplantat eller annan protetisk konstruktion där tillräcklig benvolym saknas, eller där benets kvalitet är för dålig för att medge lastbärande funktion, exempelvis permanent förankring av implantat.

PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
Case: 4092 PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/SE99/01231	06.07.1999	13.07.1998
International Patent Classification (IPC) or national classification and IPC7		
A 61 L 27/00, A 61 F 2/28		
Applicant		
Nobel Biocare AB (publ) et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
03.02.2000	14.11.2000
Name and mailing address of the IPEA/SE Patent- och registreringsverket Box 5055 S-102 42 STOCKHOLM Telex 17978 PATOREG-S Facsimile No. 08-667 72 88	Authorized officer Hélène Erikson/Els Telephone No. 08-782 25 00

Form PCT/IPEA/409 (cover sheet) (January 1998)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/SE99/01231

I. Basis of the report

1. With regard to the **elements** of the international application:*

☐ the international application as originally filed

☒ the description:
pages 1-21 , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

☒ the claims:
pages _____ , as originally filed
pages _____ , as amended (together with any statement) under article 19
pages _____ , filed with the demand
pages 16-18 , filed with the letter of 11.09.2000

☒ the drawings:
pages 1-5 , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

☐ the sequence listing part of the description:
pages _____ , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language english which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☒ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____

☐ the claims, Nos. _____

☐ the drawings, sheet/fig _____

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2 (c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item I and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/SE99/01231

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-23	YES
	Claims		NO
Inventive step (IS)	Claims	1-23	YES
	Claims		NO
Industrial applicability (IA)	Claims	1-23	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

The claimed invention relates to a preparation for restoring bone in the body of humans or animals in connection with an existing structure. It consists of an easy handleable, controllable and decomposable carrier preparation of calcium phosphate granules and a biological organic component of a biopolymer and/or lipid type. The biopolymer contains a glycoseaminoglycan, like hyaluronic acid. The lipid consists of a mixture of esterified glycerol and phospholipid. The glycerol could be di- or triglyceride or di- or triester, and the phospholipid could be either of sphingomyelin, phosphatidyl choline, vegetable oil or egg yolk or a mixture thereof. The calcium phosphate granules have a Ca/P ration of 1-2, and it contains hydroxyapatite of the form $Ca_{10}(PO_4)_3(OH)_2$. The granules have a diameter of 0,05-5 mm, and a porosity of 0-80%. The weight ratio between the granules and the lipid is in the order of 70:15 to 60:40. The preparation has been made mouldable by mixing water or body fluid into it.

The most relevant documents cited in the search report are the following:

D1 US 4 192 021 A
D2 US 5 338 772 A

.../...

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: V

D1 relates to a bone replacement material on the basis of sintered calcium phosphates and organic materials. The sintered calcium phosphates are composed of CaO and P_2O_5 in a ratio between 2:1 and 4:1, and the organic material has at least one low-molecular-weight biodegradable organic material or a least one high molecular-weight biodegradable organic material, or a mixture thereof. The polymer could be dissolved in a solvent that is soluble in blood serum. The material has a ratio of calcium phosphate to organic material of 5:1 and 1:1. The organic material could for example be a triglyceride. The calcium phosphate granules have a diameter of 0,3 to 2 mm and a porosity of 16 to 30 %.
The difference between D1 and the claimed invention is that the material in D1 has to be heated to 50-55 °C to obtain a mouldable consistency. The material in the claimed invention is of a mouldable consistency at room temperature.

In view of the above, the cited documents only disclose the general state of the art, which is not considered to be of particular relevance. Therefore, the claimed invention is considered to fulfil the requirements of novelty, inventive step and industrial applicability.

D2 also relates to an implant material based on a composite of calcium phosphate ceramic particles and bioabsorbable polymer, like polylactides and/or polyglycolides.

CLAIMS

1. A preparation for restoring bone in the body of humans or animals in connection with an existing structure, a bone implant or some other prosthetic construction, the preparation being intended to be applied in the position in connection with, for instance, a bone implant or some other prosthetic construction where there is a lack of sufficient bone volume, or where the quality of the bone is too poor, or to allow a load-carrying function, said preparation being ~~an easily handleable, controllable and decomposable carrier~~ preparation of calcium phosphate granules or a biological organic component of a biopolymer and/or lipid type c h a r a c t e r i s e d in that the preparation at its application has a mouldable consistency by admixed water or some other water-based liquid, such as body fluid.

2. A preparation as claimed in claim 1, c h a r a c t e r i s e d in that the lipid consists of a mixture of esterified glycerol and phospholipid.

3. A preparation as claimed in claim 2, c h a r a c t e r i s e d in that the esterified glycerol consists of di- and triglyceride.

4. A preparation as claimed in claim 2, c h a r a c t e r i s e d in that the esterified glycerol is a diester.

5. A preparation as claimed in claim 2, c h a r a c t e r i s e d in that the esterified glycerol is a triester.

6. A preparation as claimed in claim 1, c h a r a c t e r i s e d in that the lipid consists of a mixture of phospholipids.

7. A preparation as claimed in claim 6, c h a r a c t e r i s e d in that the phospholipid is a sphingomyelin.

8. A preparation as claimed in claim 6, c h a r a c t e r i s e d in that the phospholipid is a phosphatidyl choline.

9. A preparation as claimed in claim 1, c h a r a c t e r i s e d in that the lipid is prepared from a vegetable oil or egg yolk.

10. A preparation as claimed in any one of the preceding claims, c h a r a c t e r i s e d in that the lipid consists of at least one phospholipid and water or some other water-based liquid as carrier.

~~11. A preparation as claimed in claim 10, c h a r a c t e r i s e d~~ in that the lipid is in a lamellar floating crystalline phase.

12. A preparation as claimed in claim 10, c h a r a c t e r i s e d in that the weight ratio between lipid and water or some other water-based liquid is in the order of 1:2 to 10:1, preferably 3:2 to 4:1.

13. A preparation as claimed in claim 1, c h a r a c t e r i s e d in that the biopolymer contains a glycoseaminoglycan, for example hyaluronic acid.

14. A preparation as claimed in claim 13, c h a r a c t e r i s e d in that it consists of a free-flowing mixture of sodium hyaluronic acid and calcium phosphate granules which can be packed and then rehydrated in connection with use.

15. A preparation as claimed in claim 1, c h a r a c t e r i s e d in that the calcium phosphate granules have a Ca/P ratio which is between 1 and 2.

16. A preparation as claimed in claim 15, c h a r a c t e r i s e d in that the calcium phosphate contains hydroxyapatite of the form $Ca_{10}(PO_4)_6(OH)_2$.

17. A preparation as claimed in claim 15, c h a r a c t e r i s e d in that the calcium phosphate contains dicalcium phosphate dihydrate, octacalcium phosphate, tricalcium phosphate and/or hydroxyapatite.

18. A preparation as claimed in claim 15, c h a r a c t e r i s e d in that the calcium phosphate contains magnesium, fluorine or carbonate ions.

19. A preparation as claimed in claim 15, c h a r a c t e r i s e d in that the calcium phosphate granules have a diameter in the order of 0.05 mm to 5 mm.

20. A preparation as claimed in claim 15, c h a r a c t e r i s e d in that the calcium phosphate granules have a porosity of 0-80%.

21. A preparation as claimed in any one of the preceding claims, c h a r a c t e r i s e d in that the weight ratio between the calcium phosphate granules and the lipid is in the order of 70:15 to 60:40.

22. A preparation as claimed in any one of the preceding claims, c h a r a c t e r i s e d in that it contains tissue-promoting factors and/or factors which inhibit decomposition of tissue, for example a growth factor, such as BMP and TGF beta or parts thereof.

23. A preparation as claimed in claims 1, 14 and 22, c h a r a c t e r i s e d in that the tissue-promoting factor is added wholly or partially.

CLAIMS

1. A preparation for restoring bone in the body of humans or animals in connection with an existing structure, a bone implant or some other prosthetic construction, the preparation being intended to be applied in the position in connection with, for instance, a bone implant or some other prosthetic construction where there is a lack of sufficient bone volume, or where the quality of the bone is too poor, or to allow a load-carrying function, c h a r a c t e r i s e d in that the preparation consists of an easily handleable, controllable and decomposable carrier preparation of calcium phosphate granules or a biological organic component of a biopolymer and/or lipid type.

2. A preparation as claimed in claim 1, c h a r a c t e r i s e d in that the preparation has a mouldable consistency.

3. A preparation as claimed in claim 2, c h a r a c t e r i s e d in that the preparation has been made mouldable by admixing water or some other water-based liquid, such as body fluid.

4. A preparation as claimed in claim 2, c h a r a c t e r i s e d in that the lipid consists of a mixture of esterified glycerol and phospholipid.

5. A preparation as claimed in claim 4, c h a r a c t e r i s e d in that the esterified glycerol consists of di- and triglyceride.

6. A preparation as claimed in claim 4, c h a r a c t e r i s e d in that the esterified glycerol is a diester.

7. A preparation as claimed in claim 4, characterised in that the esterified glycerol is a triester.

8. A preparation as claimed in claim 2, characterised in that the lipid consists of a mixture of phospholipids.

9. A preparation as claimed in claim 8, characterised in that the phospholipid is a sphingomyelin.

10. A preparation as claimed in claim 8, characterised in that the phospholipid is a phosphatidyl choline.

11. A preparation as claimed in claim 2, characterised in that the lipid is prepared from a vegetable oil or egg yolk.

12. A preparation as claimed in any one of the preceding claims, characterised in that the lipid consists of at least one phospholipid and water or some other water-based liquid as carrier.

13. A preparation as claimed in claim 12, characterised in that the lipid is in a lamellar floating crystalline phase.

14. A preparation as claimed in claim 12, characterised in that the weight ratio between lipid and water or some other water-based liquid is in the order of 1:2 to 10:1, preferably 3:2 to 4:1.

15. A preparation as claimed in claim 2, characterised in that the biopolymer contains a glycoseaminoglycan, for example hyaluronic acid.

16. A preparation as claimed in claim 15, c h a r a c - t e r i s e d in that it consists of a free-flowing mixture of sodium hyaluronic acid and calcium phosphate granules which can be packed and then rehydrated in connection with use.

17. A preparation as claimed in claim 1, c h a r a c - t e r i s e d in that the calcium phosphate granules have a Ca/P ratio which is between 1 and 2.

18. A preparation as claimed in claim 17, c h a r a c - t e r i s e d in that the calcium phosphate contains hydroxyapatite of the form $Ca_{10}(PO_4)_6(OH)_2$.

19. A preparation as claimed in claim 17, c h a r a c - t e r i s e d in that the calcium phosphate contains dicalcium phosphate dihydrate, octacalcium phosphate, tricalcium phosphate and/or hydroxyapatite.

20. A preparation as claimed in claim 17, c h a r a c - t e r i s e d in that the calcium phosphate contains magnesium, fluorine or carbonate ions.

21. A preparation as claimed in claim 17, c h a r a c - t e r i s e d in that the calcium phosphate granules have a diameter in the order of 0.05 mm to 5 mm.

22. A preparation as claimed in claim 17, c h a r a c - t e r i s e d in that the calcium phosphate granules have a porosity of 0-80%.

23. A preparation as claimed in any one of the preceding claims, c h a r a c t e r i s e d in that the weight ratio between the calcium phosphate granules and the lipid is in the order of 70:15 to 60:40.

24. A preparation as claimed in any one of the preceding claims, characterised in that it contains tissue-promoting factors and/or factors which inhibit decomposition of tissue, for example a growth factor, such as BMP and TGF beta or parts thereof.

25. A preparation as claimed in claims 3, 16 and 24, characterised in that the tissue-promoting factor is added wholly or partially.

26. A method for restoring bone in the body of humans or animals in connection with an existing structure, a bone implant or some other prosthetic construction, characterised by applying an easily handleable and controllable preparation of resorbable calcium phosphate granules and a biological organic carrier of a biopolymer or lipid type in the position in the body in connection with, for example, a bone implant or some other prosthetic construction where there is a lack of sufficient bone volume, or where the quality of the bone is too poor to allow a load carrying function.

27. A method as claimed in claim 26, characterised by applying the preparation in a produced or existing cavity or defect in the bone by means of a syringe or planing appliance.

28. A method as claimed in claim 26, characterised by applying the preparation in a cavity produced for a bone implant.

29. A method as claimed in claim 26, characterised by applying the preparation in a bone defect, for example an angular defect in connection with an already installed bone implant or a cavity produced for a bone implant.

30. A method as claimed in claim 26, characterised by applying the preparation on the bone under the mucous membrane of the sinus for the purpose of increasing the vertical height of the bone ridge.

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Material for bone reconstruction

The invention relates to a preparation for restoring bone in the body of humans or animals in connection with an existing structure, a bone implant or some other prosthetic construction, as well as a method for restoring bone. The bone restoring preparation is an easily handleable and controllable preparation (composition) adapted to be applied in the position where the bone need be replaced, reinforced or built up, particularly in connection with a bone implant or some other prosthetic construction where the bone volume is insufficient, or where the quality of the bone is too poor to allow a load carrying function, for example permanent fixing of an implant.

By bone implant is in this context meant, for instance, a helical, bone-anchored implant of titanium or a titanium alloy, a so-called fixture, but also comprises other types of implant intended to be installed in bone tissue including bone from humans, especially particulated bone, but also in combination with large cortical and/or spongious bone transplants.

Background

Different types of bone grafts have been used more or less successfully to replace lost bone tissue and improve healing of bone defects for the purpose of restoring the function with and without fixed implants. In autogenous transplantation, usually from the patient's own iliac crest, it is among other things the amount of bone and the degree of resorption that affect the result of the treatment. Autologous bone grafting usually requires more than one operation to achieve a satisfactory result and causes considerable postoperative pain to the patient. In homologous bone grafting, use is made of, for instance,

demineralised bone matrix from a so-called bone bank. Tissues and structure which have been lost owing to diseases or injuries can today to some extent be replaced by prosthetic constructions which are mechanically fixed to the skeleton. Artificial hips, artificial knee-joints and dental implants are examples of how lost tissue, structure and function can be replaced by this type of construction.

Replacing lost teeth by placing dental implants in the skeleton (jaws) has a high frequency of success provided that there is a sufficient amount of bone of good quality in the close vicinity of and round the implant. After an individually adapted time of healing (3 months - 2 years), prosthetic constructions can in most cases be secured to the osseointegrated implants. Some patients have, owing to many years of lack of teeth, obtained impaired conditions than others for obtaining treatment by means of bone-secured implants. It is mainly areas in the upper jaw that have suffered most from bone destruction when the teeth are lost owing to anatomical conditions, but also areas far back in the lower jaw can have a poor quality for implant treatment.

General states of ill-health such as osteoporosis and local defects and lack of bone owing to, for instance, acute injuries, congential defects, chronical infections or local biological processes such as cysts and tumours in the jaws in most cases affect and may even make treatment with implants impossible if bone is not added or replaced in some way to increase the amount of bone locally round the implants and, thus, the initial stability.

For patients who have been treated with implants and bone replacement materials, it is important to reduce the time of osseointegration and guarantee a higher frequency of

success after installation than is possible today. For decades, experiments have been made to replace bone with organic and synthetic materials from different sources, see review, Smiler et al (1992)[1] including resorbable and non-resorbable polymers, bioactive glass, calcium phosphate compounds, calcium carbonates and naturally occurring materials such as cow bone and coral. Transplanting bone from the patient, so-called autograft, is as described above an alternative, but a relatively extensive operation which requires specialist competence, hospital treatment, an extended healing period for the transplant of at least six months and additional inconvenience and pain to the patient. Demineralised freeze-dried bone from a so-called bone bank and spongious bone with mineral from cow bone also result in an extended period of healing, a risk of immunological reactions and infectious states, but also a risk in other aspects including the frequency of success.

There is thus a great need, both with specialists and non-specialists, for being able to apply an easily accessible safe preparation for restoring bone in connection with implant treatment of patients having an insufficient volume of bone and/or too poor bone quality.

An object of the invention is to provide a preparation for restoring bone, which enables implant treatment for patients in various situations especially in areas that otherwise cannot be treated and/or have a poorer prognosis.

Calcium phosphate compounds are so-called biocompatible materials, i.e. materials which are mildly reactive with the environment, i.e. promote repair and integration of, for instance, an implant, see Jarcho[2] and Lemons[3]. The commonest form of calcium phosphate compound that is used to stimulate ossification is hydroxylapatite (HA),

$Ca_{10}(PO_4)_6(OH)_2$, but also other compounds containing calcium and phosphate ions exist and resemble the inorganic ingredients in skeleton and enamel, see Daculsi et al (1997)[4]. Stoichiometric HA, $Ca_{10}(PO_4)_6(OH)_2$, with a Ca:P ratio of 1.67 is seldom found *in vivo*. Calcium is to some extent replaced by other ions, such as magnesium, sodium, aluminium, strontium, carbonate, fluorine and chlorine, depending on, inter alia, age, food, sex etc. of the individual. HA may be present in a ceramic and in a non-ceramic form where the degree of crystallinity may vary depending on the temperature at which the calcium phosphate compound is prepared, Ricci et al (1992)[5].

Calcium phosphate compounds such as hydroxylapatite are commercially available and produced by many companies; Impladent Ltd, Holliswood, New York, USA, Asahi Optical Co, Ltd, Tokyo, Japan, Interpore Int. and Irvine, California, USA. The materials are produced with different properties such as the size of the granules/particles, the degree of resorbability and the chemical composition.

The particles/granules being resorbed do so slowly after application in the bone tissue. It is considered that from the beginning the granules physically take up room in the defect and thus allow an accelerated integrating process compared with an empty defect. While the new bone is forming, mineralising and remodelling, the granules are resorbed slowly for about 3-8 months depending on patient-related factors including the size of the defect and the age of the patient.

There are experimental studies, Hürzeler et al (1995)[6] and Wetzel et al (1995)[7] and clinical studies, Smiler et al (1992)[1], Ricci et al (1992)[5], Judy (1986)[8], Wagner (1989)[9], and Corsair (1990)[10], which demonstrate a certain effect of resorbable granules (Osteogen®) mixed with, for instance, patient blood, common salt or above all in

combination with demineralised freeze-dried bone. The possibility of making a filling in sinus maxillaris (sinus of the upper jaw) to increase the possibilities of implant fixing in dogs shows that resorbable HA granules function well and the product is suitable for use to stimulate bone formation round dental implants, Wetzel et al (1995)[7]. However, the granules are mixed with patient blood or common salt, which makes the product difficult to handle. A great drawback of this form/technique of preparation is that it is technically difficult to pass the mass of granules to the defect/cavity area. Having applied the granules, there is a great risk that blood and other body fluids from the area of the wound dilute and transport the material away from the area of application. A further drawback of uncontrolled mixing of, for instance, common salt or the patient's blood is that the risk of contamination of the preparation increases.

Alpher et al (1989)[11] have tried to solve the handling problems. However this reference shows that a mouldable hydroxylapatite preparation based on phospholipids and stearic acid generally does not stimulate the formation of new bone or bone growth, but instead the results indicate a reduction of the bone formation.

Lipids can be divided into different classes. Triglycerides are the most frequent class of lipids and are an important depot of energy in cells. Triglycerides are either built up or decomposed in the body by the intermediary of diglycerides from or into monoglycerides and fatty acids. The body also contains different types of membrane lipids, for example phosphatidyl choline, phosphatidyl ethanolamine, sphingomyelin, cholesterol, mono- and digalactosyldiacylglycerol.

Phospholipids can be prepared fully synthetically but also be cleaned of biological raw materials such as

plants or animals. Examples of raw materials are egg yolk, vegetable oils such as soybean oil, rapeseed oil.

It is also preferred for the preparation to contain antioxidants selected according to known principles or naturally occurring. An example of an advantageous antioxidant in this case can be tocopherol. Liposomes consist of a spherical shell of amphiphilic lipids containing an aqueous phase. The potential of the lipid vesicles as carrier of drugs has been studied and described in a number of articles. Huang et al[12] have suggested that negatively charged liposomes can affect the mineralising process of newly formed bone. This concept was tested in a defect model in miniature swine, however, without any effect. Bone formation between uncoated and liposome-coated calcium phosphate compounds was compared and the uncoated calcium phosphate compounds were surrounded by more bone and riper bone than the liposome-coated ones. In an experimental study by Raggio et al (1986)[13], the authors show that complex acid lipids affect the precipitation of hydroxylapatite mineral in a physiological environment.

Recently some research has focused on the application of introducing exogenic molecules into cells by means of lipid complexes. These new lipids have an important clinical application for drug delivery and gene therapy. Since the lipids can be tailored to have different physical properties, the application may vary, Ashley et al 1996)[14] and Barber et al (1996)[15].

Different systems based on calcium phosphate granules and lipid carriers are described in literature, see for instance EP 0 429 419 which discloses a system where calcium phosphate, especially hydroxylapatite, is used as bone substitute material. In this case, use is made of a monoglyceride-based carrier, which may cause a drawback

in implant treatment since preliminary studies indicate that encapsulation may occur, which in turn can have a negative effect on implant integration.

Various systems for the release of pharmaceutical preparations containing bioceramic granules and lipid have also been described in literature, see for instance JP 2,631,890. As examples of different carriers for drugs and molecules that are to be released in bone tissue, mention can be made of collagen, lipids, polymers (for instance PLA/PGA and hyaluronic acid) and ceramics.

A large number of studies demonstrate that mineral deposition in cartilage that is being calcified is only found in vesicles containing phosphatidyl serine and alkaline phosphatase, and that the endocondral calcification process in the growth plate in the epiphysis can be mediated by these. Matrix vesicles and the negatively charged phospholipids therein seem to be involved in the initial formation of calcium hydroxylapatite crystals by way of the interaction between calcium and phosphate ions with phosphatidyl serine in the formation of phospholipid:calcium:phosphate ion complex, Boyan et al (1989)[16].

It is mainly the systemically blood calcium controlling hormones which in different ways control bone cells and, thus, keep the bone mass of the body in equilibrium. In recent years, many studies have been made which indicate that certain biopolymers of the polypeptide type produced by bone cells themselves and/or blood cells from bone marrow or in inflammation after, for instance, trauma, have an important and probably a more immediate importance for activating the individual cells in connection with the bone formation process.

Bone formation and bone resorption are connected to each other. Systemically and locally produced factors control

the processes. Many of the growth factors may have different effect on different cells. For instance, PTH and vitamin D can stimulate bone resorption and remodelling by means of the bone-forming cells, cf. Nijweide et al (1986)[17]. On the other hand, the bone-forming cells can be stimulated by TGF beta released of matrix during the bone resorption process, cf. Pfeilschifter et al (1990)[18].

The growth factors and cytokines that are produced by bone cells may have an autocrine or paracrine effect. Examples hereof are: TGF, IGF-I and IGF-II, Beta2 Microglobulin, PDGF and CSFs. Thrombocyte-derivated factors such as TGF, PDGF and EGF, but also interleukins, TNFs and Interferon gamma are factors of hematologic origin which have effect on the bone-forming cells. Growth factors which are stored in the bone matrix are the largest reservoir for growth factor activity. The factors stored are, as mentioned above, TGF, IGF-I and IGF-II, Beta2 Microglobulin, PDGF, but also FGF. Bone morphogenetic proteins, BMP, and osteogenine belong to the TGF family. BMP is usually combined with decalcified bone matrix and collagen, cf. Sampath and Reddi (1981)[19] and Saito et al (1994)[20]. Kuboki et al (1995)[21] have proved that BMP induces only bone better if the HA carrier consists of particles which are porous compared with non-porous.

In a newly published experimental study by Urist et al (1997)[22], different systems for administration of the growth factor BMP-2 and its effect on bone formation were investigated. The authors suggest that lipids extracted from bone can function well as a carrier of bone-stimulating peptides in the bone formation process.

Other molecules or ions which can bind strongly to crystal surfaces are, for example, bisphosphonates which can affect osteoblasts and thus dissolution of calcium phos-

phate compounds in the skeleton, cf. Ebrahimpour et al (1995)[23].

According to the invention, the preparation for restoring bone is a mixture of resorbable calcium phosphate granules and/or a carrier of a biopolymer or lipid type, where the lipid contains an esterified fatty acid selected from the group consisting of triglycerides, diglycerides or phospholipids or combinations thereof. The invention aims at overcoming the difficulties described above and constituting a preparation which easily and in a repetitive manner can be used in connection with bone implants. More specifically, the inventive material is intended to withstand dilution and transporting away from the area of application. Such a mixture can be given the "correct" consistency depending on the type of application, it can be made, for example, mouldable, and it is easy to handle, control and apply.

A desirable weight ratio between calcium phosphate and phospholipid is in the order of 70:15 to 60:40. A desirable weight ratio between phospholipid and water or other water-based liquids is in the order of 1:2 to 10:1, preferably 3:2 to 4:1.

With reference to the water-based liquid that is used to make the preparation mouldable, pure water, an isotonic saline solution or a pharmaceutically acceptable solution are preferred. In some cases when the preparation is being produced in situ, body fluids including blood can be used.

The invention will now be described in more detail in connection with some applications of bone implants where the surrounding bone tissue need to be reinforced and/or built-up,

Fig. 1 showing a case where the bone forms a far too loose, load-carrying network,

Fig. 2 showing a case where the lateral bone volume is insufficient,

Fig. 3 showing a case having angular and/or too narrow bone ridges,

Fig. 4 showing a case where the vertical bone height is insufficient, and

Fig. 5 being a picture of a preparation applied in rabbit bone.

As mentioned by way of introduction, the preparation for restoring bone consists of a mixture of resorbable calcium phosphate granules and a carrier of a biopolymer or lipid type. To be applied in connection with a bone implant and be kept in the area of application, it is important for the mixture to be mouldable and to have the correct consistency. If the particles are transported away from the area of application, they could cause irritation or complications in other positions in the body.

The calcium phosphate granules should have a Ca/P ratio of between 1 and 2. The granules should have an average diameter of 0.05 - 5 mm and a micro/macro porosity of 0-80%.

In a study made by Neo et al (1992)[24], the interface between bioactive ceramics and bone was studied by using scanning and transmission electron microscopy. Calcium phosphate granules having an average diameter of 0.1 to 0.3 mm were studied and characterised in respect of resorbability. After 8 weeks, the non-resorbable granules were connected with bone by a thin Ca-P-rich layer con-

sisting of fine apatite crystals, however, different from those in bone in respect of shape, size and orientation. The resorbable granules, however, had direct contact with the bone. The surface of the granules was coarser owing to degradation, and analyses demonstrated that bone grew into the smallest surface irregularities. In another study made by Kitsugi et al[25], four types of calcium phosphate ceramics were compared. The Ca/P ratio was in this case 1, 1.5 and 1.66 and the size of the particles (granules) varied between 0.15 and 0.3 mm. Observations made by transmission electron microscopy showed that the Ca/P ratio did not affect the connection and contact between the particles and the surrounding bone.

According to the invention it is necessary to have a carrier for the resorbable calcium phosphate granules, which may consist of a biopolymer or a lipid containing esters of fatty acids, such a triglyceride, diglyceride, or phospholipids or combinations thereof, for instance some of the lipids described in WO 95/34287.

Preferably, the calcium phosphate granules are distributed in a lamellar, liquid crystalline phase which contains at least one phospholipid and forms either in the body or earlier.

Example 1

The following Example describes a test for the handleability of a HA granule/lipid mixture, a phospholipid, (Epikuron 200 supplied by Lucas Mayer) being mixed with HA granules (in this case Apaceram by Pentax) and ethanol, series A samples 1-8. These samples have then been freeze-dried to a constant weight. After freeze-drying, the samples have a composition which is evident from the Table below.

In series B, samples 9-17 have been mixed according to the Table below, whereupon the mouldability has been assessed. The samples were made by 0.9% by weight NaCl in water (physiological salt solution) being weighed and mixed with phospholipid (PC), whereupon hydroxyapatite granules were added. All samples in this series contained 70% by weight of HA and the amount of granules has been kept constant whereas the ratio of phospholipid to water has been varied. The samples that contain only two components PC and granules, as well as water and granules, did not have a satisfactory handleability.

Series A, samples 1-8

SAMPLE No.	PC %	HA %	CONSISTENCY
1	10.1	89.9	crumbly
2	15.1	84.9	crumbly
3	20.1	79.9	crumbly
4	25.0	75.0	slightly mouldable
5	30.1	69.9	mouldable
6	35.1	64.9	mouldable
7	40.0	60.0	mouldable
8	45.0	55.0	mouldable

Series B, samples 9-17

SAMPLE No.	PC %	0.9% NACL ISG	HA %	CONSISTENCY
9	3.2	27.0	69.9	crumbly
10	6.0	24.0	70.0	crumbly
11	9.2	20.9	69.9	crumbly, slightly mouldable
12	12.0	18.1	69.9	crumbly, slightly mouldable
13	15.0	15.2	69.9	slightly mouldable
14	18.0	12.0	70.0	mouldable
15	21.1	9.1	69.8	mouldable
16	24.1	6.1	69.8	mouldable
17	27.0	3.0	70.0	crumbly, slightly mouldable

The weight ratio between the calcium phosphate component and the lipid and the admixture of water are determined by the requirement that the preparation should be easily handleable and mouldable. Preferably, the weight ratio between calcium phosphate and phospholipid should be within the range 70:15 to 60:40. The weight ratio of phospholipid to water or some other water-based liquid should be in the range 1:2 to 10:1, preferably 3:2 to 4:1.

Example 2

Mouldable preparations were produced by mixing 0.21 g phospholipid (1,2-dioleyl-sn-glycero-3-phosphocholine, Avanti Polar Lipids, Inc.) with 0.12 g 0.9% physiological salt solution. After one hour, 0.71 g hydroxyapatite granules (OsteoGen® (HA Resorb)®, Impladent Ltd.) was added. The preparation was mixed to a mouldable consistency and was easy to pack in an applicator.

The preparation was evaluated in respect of handleability and applicability in a defect model where circular defects having a diameter of 4 mm had been created in the lower part of the leg (tibia) on adult New Zealand White rabbits. The evaluation shows that the preparation was handleable and easy to apply in the defect area with a modified syringe. The preparation was kept together and was not affected by the relatively strong flow of blood from the area of the wound. Fig. 5 is a picture of the applied preparation inserted in the rabbit bone. An evaluation is now made in respect of the amount of newly formed bone (histomorphometrically) in the defect area, and the result will be compared in pairs with an untreated defect produced in an identical manner.

Instead of a lipid of the type described above, the carrier can consist of a biopolymer, a proteoglycan, a glycoseaminoglycan, for instance hyaluronic acid. Hyaluronic acid is an anionic polysaccharide, composed of repetitive disaccharide units of beta-1-4-glucoronate-beta-1-3-N--acetyl-glucoseamine and is part of the extracellular matrix. Hyaluronic acid serves as lubricant in joints, is present in large quantities in connective tissue and is present in abundance in the eye. In a study investigating whether phagocytable particles of hydroxyapatite have a detrimental effect on bone formation, sodium hyaluronic acid has been used as a carrier solution for hydroxyapatite, cf. Wang J-S et al (1994)[26].

The following formulation of hyaluronic acid and hydroxyapatite can be used as bone substitute material according to the invention. A freeze-dried mixture of sodium hyaluronic acid (Healon®) and hydroxyapatite (65:35%w/w) swells in the presence of water and thus is easy to form and handle. The freeze-dried mixture of sodium hyaluronic acid and hydroxyapatite is easily packed in an applicator and/or a modified syringe, which is rehydrated with

water, an aqueous solution or body fluid close to the application so as to form a mouldable hydroxyapatite formulation for restoring bone.

The bone restoring material according to the invention or the granules themselves can advantageously be combined with a cell-stimulating substance which is known per se, for instance growth factors such as BMP and relating to the TGF beta family. Bone-stimulating substances and molecules transmit signals and affect cells and cellular activities in bone. Proteins and polypeptides are examples of substances which have been found to have a bone-stimulating effect in different ways, mainly locally. In addition to the fact that the preparation can be mixed with a bone implant from the patient himself, calcium phosphates granules can be added, containing bone-stimulating substances or parts thereof.

Depending on the properties of the bone-inducing or tissue-promoting factors, some of them exist in an active and an inactive form, respectively, and need be combined with different types of carrier to induce bone and cartilage *in vivo*. First this was explained by the fact that the growth factor diffused out too fast and was not kept in place sufficiently long for an effect to be achieved. Later studies have proved that the carrier also serves as support and substrate for cells to adhere to and diffuse on since bone formation can occur only on a surface or substrate, cf. Kuboki et al (1995)[21]. Common examples of a carrier for BMP are decalcified bone matrix and collagen, cf. Sampath and Reddi (1981)[19] and Saito et al (1994)[20]. Kuboki et al (1995)[21] have demonstrated that BMP induces only bone if the HA carrier consists of particles that are porous. Tsuruga et al (1997)[27] present theories about cell differentiation and bone formation being strongly dependent on the carrier in its capacity as substrate surface and microenvironment. According to the invention

it is thus suggested that the mouldable calcium phosphate granule-lipid mixture is allowed to constitute a carrier for, for example, growth factors.

Some examples of situations in which the invention can be applied will be illustrated below with reference to Figs 1-4:

Fig. 1

A. After boring (and prethreading) of the bone, B. the preparation is applied in the produced bore by means of a syringe or an inserting appliance. The preparation is pressed against the walls of the cavity and, owing to the bone architecture consisting of no network at all or a loose network, the penetration of the preparation peripherally outwards in the tissue is made possible. C. A helical bone implant (fixture) is installed according to a standard procedure and, after integration, the bone round the fixture has been remodelled and assumed a more lamellar structural appearance and thus is also capable of absorbing loads in a more advantageous manner.

Fig. 2

A. After boring (and prethreading), B. the preparation applied in the produced bore by means of, for instance, a syringe or a similar inserting appliance. The preparation is applied in the defect and, owing to the consistency of the preparation, the granules remain in the application area and thus enable new formation of bone in the defect area. C. The fixture can be installed according to a standard procedure directly by bicortical fixing, D. but also after a short or long period of integration when the

bone in the area has been converted and assumed a more lamellar structural and cortical appearance.

Fig. 3

A. After boring (and prethreading), B1. the preparation is applied before the fixture is installed or B2. after the fixture has been installed in the case of unsatisfactory vertical and horizontal bone volume, for instance angular defects and/or too narrow bone ridges. These two situations are examples of situations where part of the fixture initially does not have a satisfactory contact with the surrounding bone tissue. C. After integrating, the healing process has produced the normal bone architecture in the area.

Fig. 4

After toothlessness in the upper jaw, the sinus has in many cases expanded while at the same time the bone ridge has been resorbed. This means that the vertical height that is required for the anchoring of fixtures is insufficient.

Alternative I A. After boring (and prethreading), B. the preparation is applied by means of a modified syringe or some other appliance under the mucous membrane of the sinus. C. The fixture is installed and after unaffected integrating the preparation results in new bone forming in connection with and in contact with the fixture.

Alternative II In a sinus lift, the preparation can also be applied laterally, i.e. communication with the sinus occurs by way of a laterally produced defect, whereupon the mucous membrane of the sinus is lifted and

parts of the bottom of the sinus can be filled with the preparation.

Fig. 5

The picture illustrates a preparation according to Example 2 above applied in a rabbit bone, as described above.

CLAIMS

1. A preparation for restoring bone in the body of humans or animals in connection with an existing structure, a bone implant or some other prosthetic construction, the preparation being intended to be applied in the position in connection with, for instance, a bone implant or some other prosthetic construction where there is a lack of sufficient bone volume, or where the quality of the bone is too poor, or to allow a load-carrying function, c h a r a c t e r i s e d in that the preparation consists of an easily handleable, controllable and decomposable carrier preparation of calcium phosphate granules or a biological organic component of a biopolymer and/or lipid type.

2. A preparation as claimed in claim 1, c h a r a c t e r i s e d in that the preparation has a mouldable consistency.

3. A preparation as claimed in claim 2, c h a r a c t e r i s e d in that the preparation has been made mouldable by admixing water or some other water-based liquid, such as body fluid.

4. A preparation as claimed in claim 2, c h a r a c t e r i s e d in that the lipid consists of a mixture of esterified glycerol and phospholipid.

5. A preparation as claimed in claim 4, c h a r a c t e r i s e d in that the esterified glycerol consists of di- and triglyceride.

6. A preparation as claimed in claim 4, c h a r a c t e r i s e d in that the esterified glycerol is a diester.

7. A preparation as claimed in claim 4, c h a r a c - t e r i s e d in that the esterified glycerol is a triester.

8. A preparation as claimed in claim 2, c h a r a c - t e r i s e d in that the lipid consists of a mixture of phospholipids.

9. A preparation as claimed in claim 8, c h a r a c - t e r i s e d in that the phospholipid is a sphingomyelin.

10. A preparation as claimed in claim 8, c h a r a c - t e r i s e d in that the phospholipid is a phosphatidyl choline.

11. A preparation as claimed in claim 2, c h a r a c - t e r i s e d in that the lipid is prepared from a vegetable oil or egg yolk.

12. A preparation as claimed in any one of the preceding claims, c h a r a c t e r i s e d in that the lipid consists of at least one phospholipid and water or some other water-based liquid as carrier.

13. A preparation as claimed in claim 12, c h a r a c - t e r i s e d in that the lipid is in a lamellar floating crystalline phase.

14. A preparation as claimed in claim 12, c h a r a c - t e r i s e d in that the weight ratio between lipid and water or some other water-based liquid is in the order of 1:2 to 10:1, preferably 3:2 to 4:1.

15. A preparation as claimed in claim 2, c h a r a c - t e r i s e d in that the biopolymer contains a glycose-aminoglycan, for example hyaluronic acid.

16. A preparation as claimed in claim 15, c h a r a c - t e r i s e d in that it consists of a free-flowing mixture of sodium hyaluronic acid and calcium phosphate granules which can be packed and then rehydrated in connection with use.

17. A preparation as claimed in claim 1, c h a r a c - t e r i s e d in that the calcium phosphate granules have a Ca/P ratio which is between 1 and 2.

18. A preparation as claimed in claim 17, c h a r a c - t e r i s e d in that the calcium phosphate contains hydroxyapatite of the form $Ca_{10}(PO_4)_6(OH)_2$.

19. A preparation as claimed in claim 17, c h a r a c - t e r i s e d in that the calcium phosphate contains dicalcium phosphate dihydrate, octacalcium phosphate, tricalcium phosphate and/or hydroxyapatite.

20. A preparation as claimed in claim 17, c h a r a c - t e r i s e d in that the calcium phosphate contains magnesium, fluorine or carbonate ions.

21. A preparation as claimed in claim 17, c h a r a c - t e r i s e d in that the calcium phosphate granules have a diameter in the order of 0.05 mm to 5 mm.

22. A preparation as claimed in claim 17, c h a r a c - t e r i s e d in that the calcium phosphate granules have a porosity of 0-80%.

23. A preparation as claimed in any one of the preceding claims, c h a r a c t e r i s e d in that the weight ratio between the calcium phosphate granules and the lipid is in the order of 70:15 to 60:40.

24. A preparation as claimed in any one of the preceding claims, c h a r a c t e r i s e d in that it contains tissue-promoting factors and/or factors which inhibit decomposition of tissue, for example a growth factor, such as BMP and TGF beta or parts thereof.

25. A preparation as claimed in claims 3, 16 and 24, c h a r a c t e r i s e d in that the tissue-promoting factor is added wholly or partially.

26. A method for restoring bone in the body of humans or animals in connection with an existing structure, a bone implant or some other prosthetic construction, c h a r a c t e r i s e d by applying an easily handleable and controllable preparation of resorbable calcium phosphate granules and a biological organic carrier of a biopolymer or lipid type in the position in the body in connection with, for example, a bone implant or some other prosthetic construction where there is a lack of sufficient bone volume, or where the quality of the bone is too poor to allow a load carrying function.

27. A method as claimed in claim 26, c h a r a c t e r i s e d by applying the preparation in a produced or existing cavity or defect in the bone by means of a syringe or planing appliance.

28. A method as claimed in claim 26, c h a r a c t e r i s e d by applying the preparation in a cavity produced for a bone implant.

29. A method as claimed in claim 26, c h a r a c t e r i s e d by applying the preparation in a bone defect, for example an angular defect in connection with an already installed bone implant or a cavity produced for a bone implant.

30. A method as claimed in claim 26, characterised by applying the preparation on the bone under the mucous membrane of the sinus for the purpose of increasing the vertical height of the bone ridge.

Fig. 1

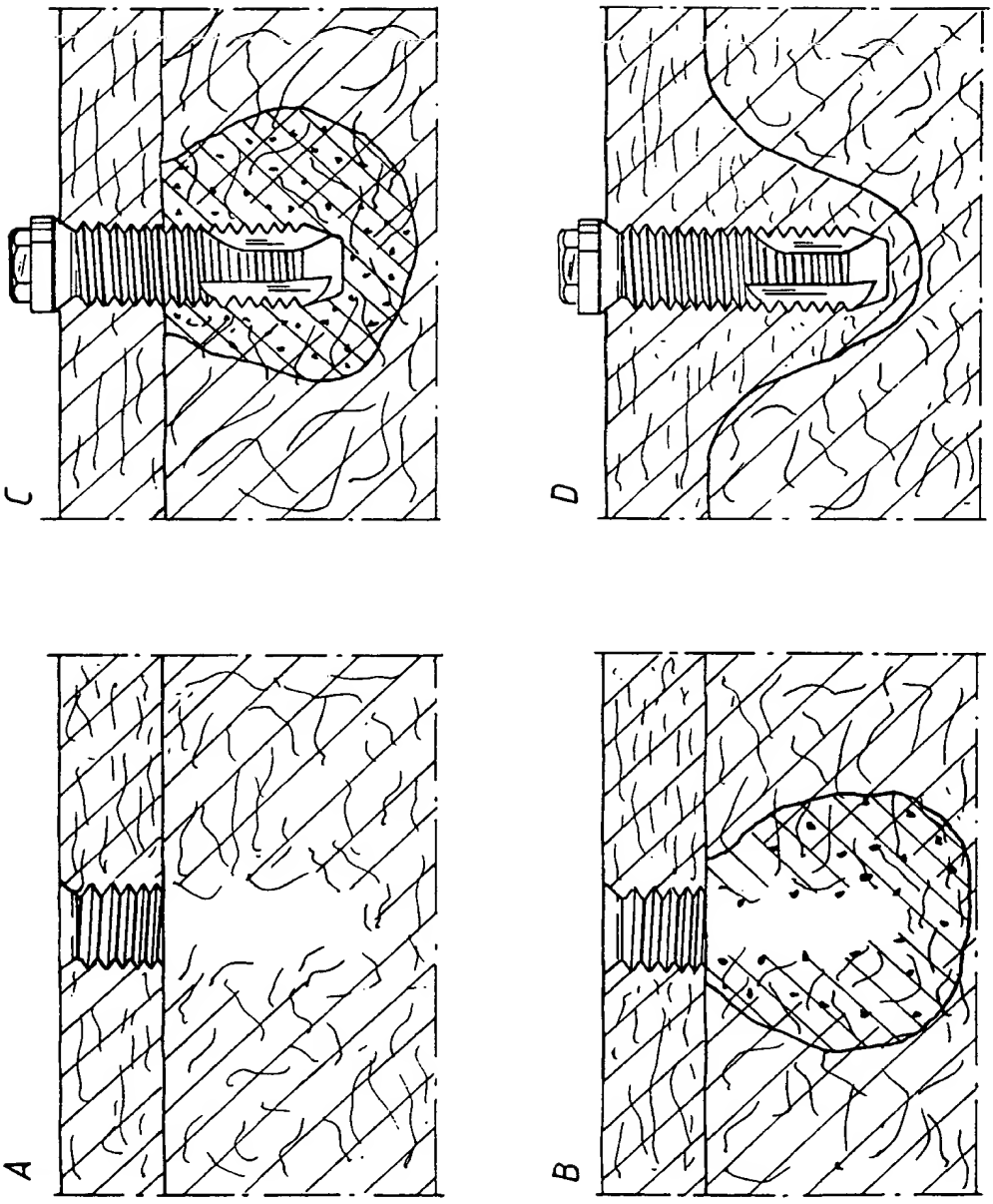


Fig. 2

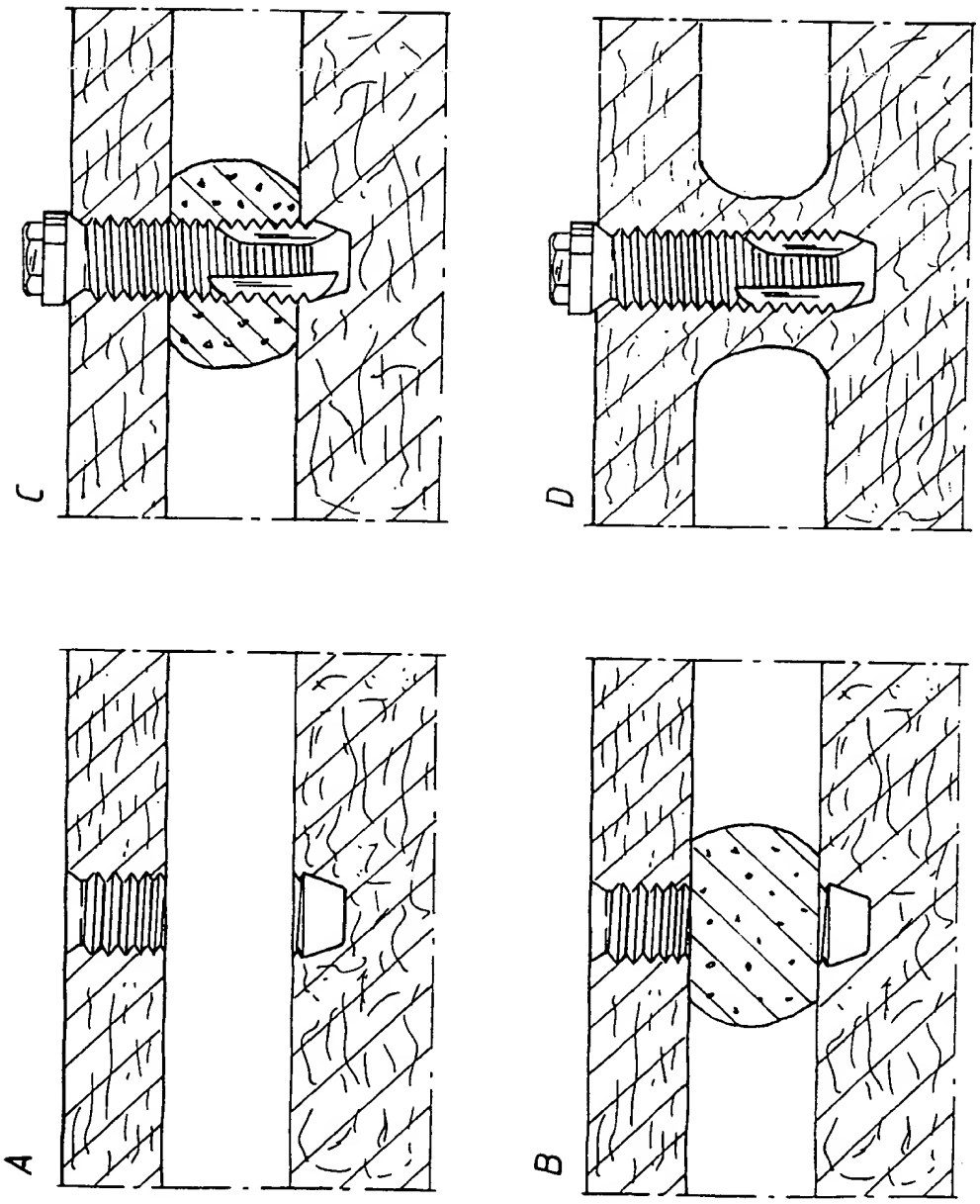


Fig. 2

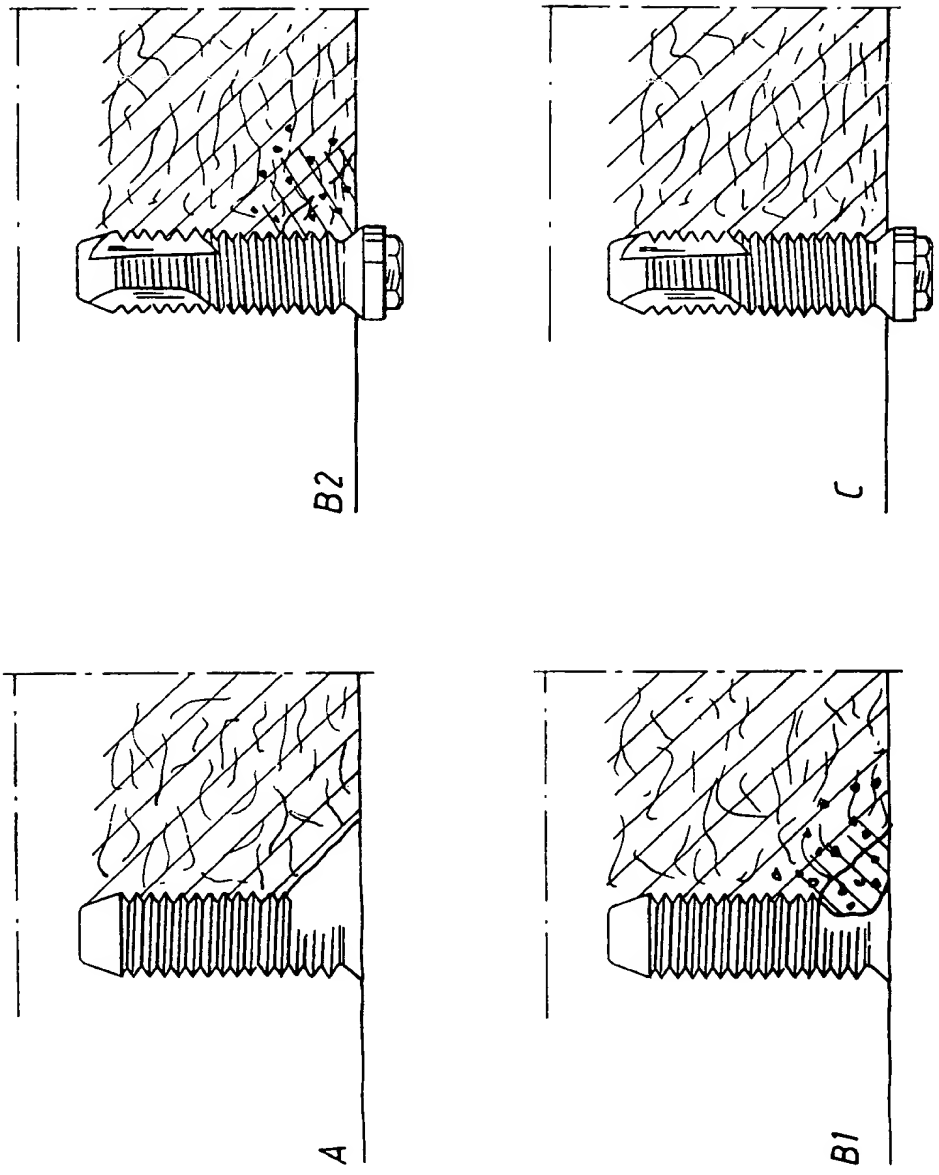


Fig 4

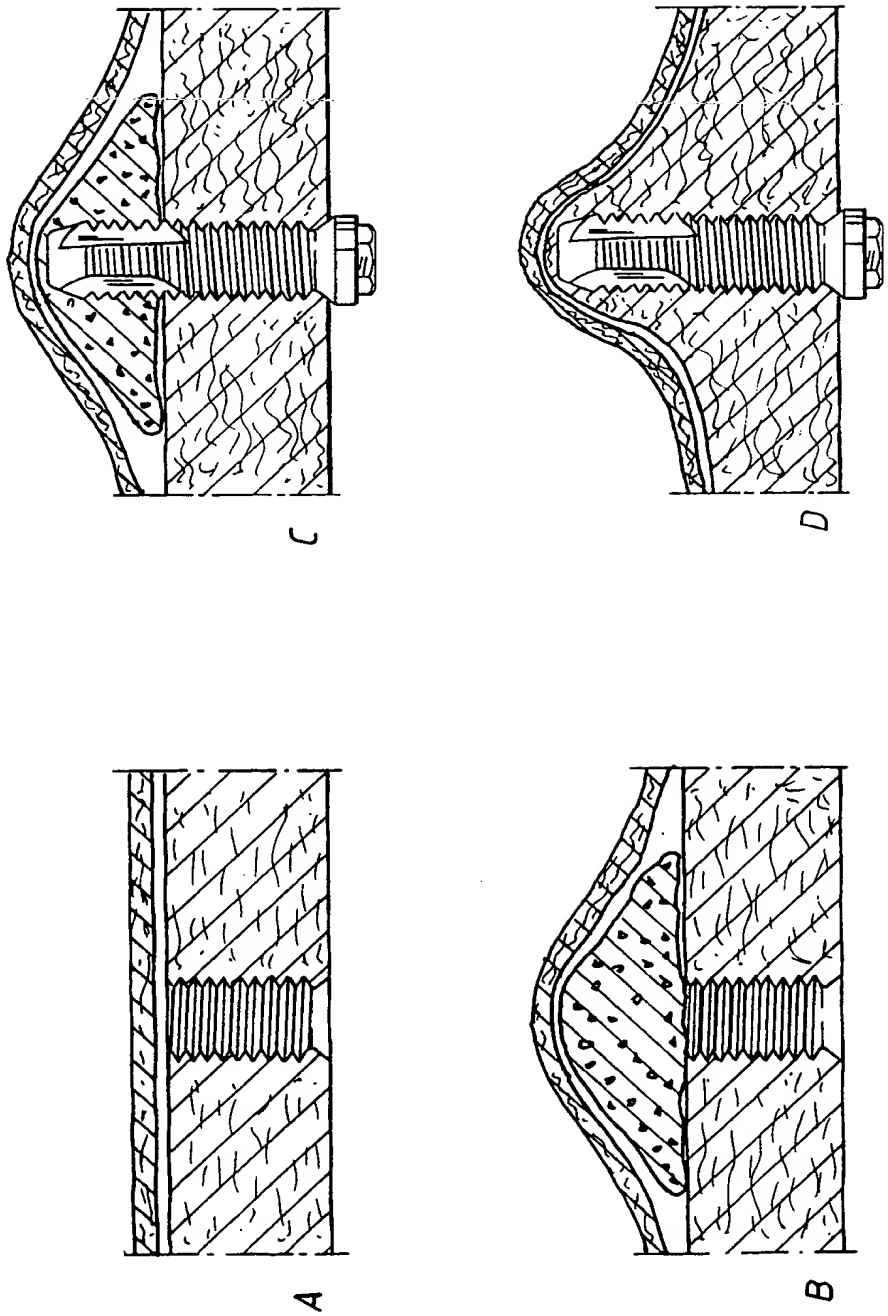
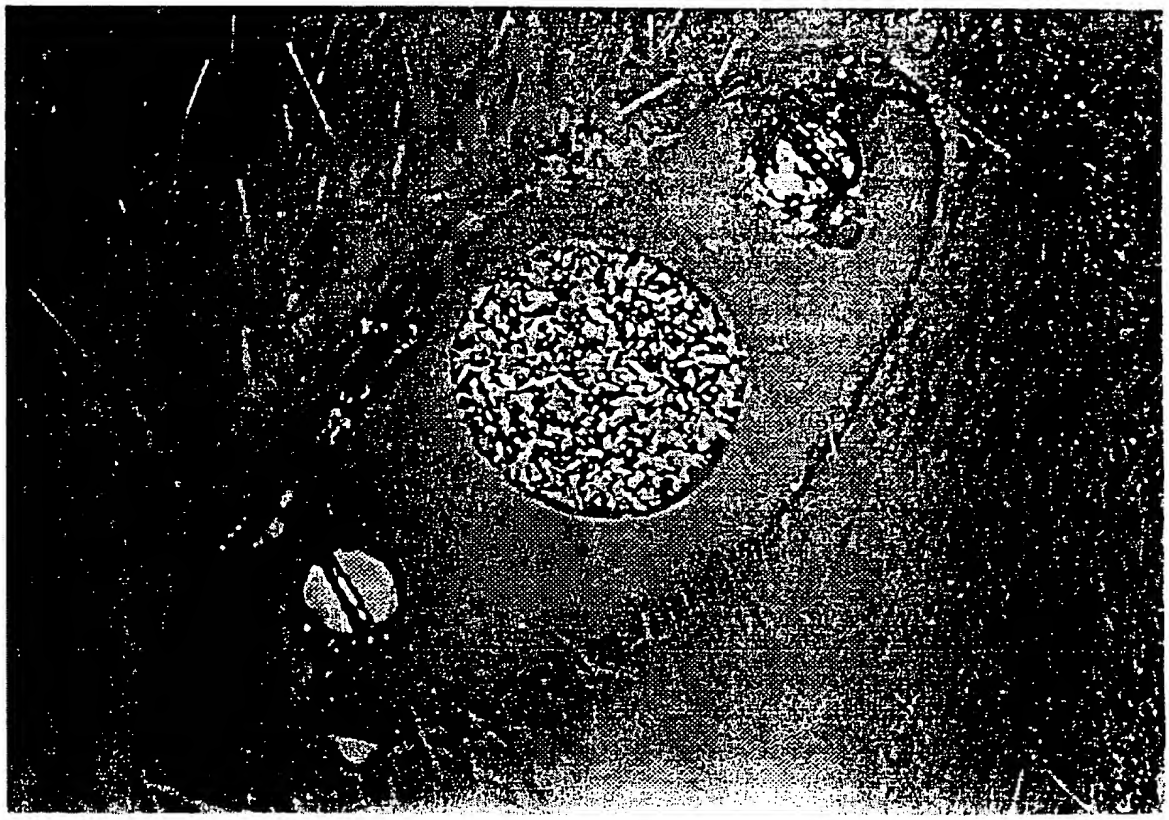


Fig. 5



INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 99/01231

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: A61L 27/00, A61F 2/28
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A61L, A61F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4192021 A (HEINRICH DEIBIG ET AL), 11 March 1980 (11.03.80), claims, abstract	1-25
X	US 5338772 A (HANS-JÖRG BAUER ET AL), 16 August 1994 (16.08.94), claims, abstract	1-25
A	US 5092888 A (OSAMU IWAMOTO ET AL), 3 March 1992 (03.03.92), claims, abstract	1-25
A	WO 9745147 A1 (ONTARIO INC.), 4 December 1997 (04.12.97), claims, abstract	1-25

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 October 1999	09 -11- 1999
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Jack Hedlund/ELY Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 99/01231

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5769897 A (ANTON HÄRLE), 23 June 1998 (23.06.98), claims, abstract ---	1-25
A	SE 464912 B (BIOAPATITE AB), 1 July 1991 (01.07.91), claims, abstract --- --------	1-25

INTERNATIONAL SEARCH REPORT

International application No.
SE99/01231

Box I **Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 26-30
because they relate to subject matter not required to be searched by this Authority, namely:

See PCT Rule 39.1 (iv): Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Box II **Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

28/09/99

International application No.
PCT/SE 99/01231

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 4192021 A	11/03/80	AT 352867 B	10/10/79
		CH 632158 A	30/09/82
		DE 2620890 A,B,C	17/11/77
		FR 2350826 A,B	09/12/77
		GB 1562758 A	19/03/80
		IT 1085566 B	28/05/85
		NL 7704659 A	15/11/77
		DE 2620891 A,B,C	17/11/77
US 5338772 A	16/08/94	AT 174225 T	15/12/98
		DE 4120325 A	24/12/92
		DE 59209585 D	00/00/00
		EP 0519293 A,B	23/12/92
		ES 2127735 T	01/05/99
US 5092888 A	03/03/92	DE 4016135 A	22/11/90
		JP 1917090 C	23/03/95
		JP 2307845 A	21/12/90
		JP 6045487 B	15/06/94
WO 9745147 A1	04/12/97	AU 2759397 A	05/01/98
		CA 2252860 A	04/12/97
		EP 0906128 A	07/04/99
US 5769897 A	23/06/98	NONE	
SE 464912 B	01/07/91	SE 8903538 A	26/04/91